EXHIBIT J – PART 3

Open Letter from World Scientists to All Governments Concerning Genetically Modified Organisms (GMOs)

- The scientists are extremely concerned about the hazards of GMOs to biodiversity, food safety, human and animal health, and demand a moratorium on environmental releases in accordance with the precautionary principle.
- They are opposed to GM crops that will intensify corporate monopoly, exacerbate inequality and prevent the essential shift to sustainable agriculture that can provide food security and health around the world.
- They call for a ban on patents of life-forms and living processes which threaten food security, sanction biopiracy of indigenous knowledge and genetic resources and violate basic human rights and dignity.
- They want more support on research and development of non-corporate, sustainable agriculture that can benefit family farmers all over the world.

Previous versions of this letter were submitted to many governments and international forums including:

- **World Trade Organization** Conference in Seattle (November 30 – Dec. 2, 1999)
- **UN Biosafety Protocol Meeting** in Montreal (24 – 28, Jan. 2000)
- **UN Commission on Sustainable Development** Conference on Sustainable Agriculture in New York (April 24-May 5, 2000)
- **UN Convention on Biological Diversity** Conference in Nairobi (May 16-24, 2000)
- **United States Congress** (29 June, 2000)

Signed by 828 scientists from 84 different countries, including:

Dr. David Bellamy, Biologist and Broadcaster, London, UK
Prof. Liebe Cavalieri, Mathematical Ecologist, Univ. Minnesota, USA
Dr. Thomas S. Cox, Geneticist, US Dept. of Agriculture (retired), India
Dr. Tewolde Egziabher, Spokesperson for African Region, Ethiopia
Dr. David Ehrenfeld, Biologist/Ecologist, Rutgers University, USA
Dr. Vladimir Zajac, Oncovirologist, Genetisist, Cancer Reseach Inst, Czech Republic
Dr. Brian Hursey, ex FAO Senior Officer for Vector Borne Diseases, UK
Prof. Ruth Hubbard, Geneticist, Harvard University, USA
Prof. Jonathan King, Molecular Biologist, MIT, Cambridge, USA
Prof. Gilles-Eric Seralini, Laboratoire de Biochimie & Moleculaire, Univ. Caen, France
Dr. David Suzuki, Geneticist, David Suzuki Foundation, Univ. British Columbia, Canada
Dr. Vandana Shiva, Theoretical Physicist and Ecologist, India
Dr. George Woodwell, Director, Woods Hole Research Center, USA
Prof. Oscar B. Zamora, Agronomist, U. Philippines, Los Banos, Philippines

add your name to the list!

1.9.2000

Open Letter from World Scientists to All Governments

Summary

We, the undersigned scientists, call for the immediate suspension of all environmental releases of GM crops and products, both commercially and in open field trials, for at least 5 years; for patents on living processes, organisms, seeds, cell lines and genes to be revoked and banned; and for a comprehensive public enquiry into the future of agriculture and food security for all.

Patents on life-forms and living processes should be banned because they threaten food security, sanction biopiracy of indigenous knowledge and genetic resources, violate basic human rights and dignity, compromise

healthcare, impede medical and scientific research and are against the welfare of animals.

GM crops offer no benefits to farmers or consumers. Instead, many problems have been identified, including yield drag, increased herbicide use, erratic performance, and poor economic returns to farmers. GM crops also intensify corporate monopoly on food, which is driving family farmers to destitution, and preventing the essential shift to sustainable agriculture that can guarantee food security and health around the world

The hazards of GMOs to biodiversity and human and animal health are now acknowledged by sources within the UK and US Governments. Particularly serious consequences are associated with the potential for horizontal gene transfer. These include the spread of antibiotic resistance marker genes that would render infectious diseases untreatable, the generation of new viruses and bacteria that cause diseases, and harmful mutations which may lead to cancer.

In the Cartegena Biosafety Protocol negotiated in Montreal in January 2000, more than 130 governments have pledged to implement the precautionary principle and to ensure that biosafety legislations at the national and international levels take precedence over trade and financial agreements at the World Trade Organization.

Successive studies have documented the productivity and the social and environmental benefits of sustainable, low-input and organic farming in both North and South. They offer the only practical way of restoring agricultural land degraded by conventional agronomic practices, and empower small family farmers to combat poverty and hunger.

We urge the US Congress to reject GM crops as both hazardous and contrary to the interest of family farmers; and to support research and development of sustainable agricultural methods that can truly benefit family farmers all over the world.

We, the undersigned scientists, call for the immediate suspension of all environmental releases of GM crops and products, both commercially and in open field trials, for at least 5 years; for patents on living processes, organisms, seeds, cell lines and genes to be revoked and banned; and for a comprehensive public enquiry into the future of agriculture and food security for all.

1 Patents on life-forms and living processes should be banned because they threaten food security, sanction biopiracy of indigenous knowledge and genetic resources, violate basic human rights and dignity, compromise healthcare, impede medical and scientific research and are against the welfare of animals(1). Life-forms such as organisms, seeds, cell lines and genes are discoveries and hence not patentable. Current GM techniques which exploit living processes are unreliable, uncontrollable and unpredictable, and do not qualify as inventions. Furthermore, those techniques are inherently unsafe, as are many GM organisms and products.

2. It is becoming increasingly clear that current GM crops are neither needed nor beneficial. They are a dangerous diversion preventing the essential shift to sustainable agricultural practices that can provide food security and health around the world.

3. Two simple characteristics account for the nearly 40 million hectares of GM crops planted in 1999(2). The majority (71%) are tolerant to broad-spectrum herbicides, with companies engineering plants to be tolerant to their own brand of herbicide, while most of the rest are engineered with bt-toxins to kill insect pests. A university-based survey of 8200 field trials of the most widely grown GM crops, herbicide-tolerant soya beans - revealed that they yield 6.7% less and required two to five times more herbicides than non-GM varieties(3). This has been confirmed by a more recent study in the University of Nebraska(4). Yet other problems have been identified: erratic performance, disease susceptibility(5), fruit abortion(6) and poor economic returns to farmers(7).

4. According to the UN food programme, there is enough food to feed the world one and a half times over. While world population has grown 90% in the past 40 years, the amount of food per capita has increased by 25%, yet one billion are hungry(8). A new FAO report confirms that there will be enough or more than enough food to meet global demands without taking into account any yield improvementsthat might result from GM crops well into 2030 (9). It is on account of increasing corporate monopoly operating under the globalised economy that the poor are getting poorer and hungrier(10). Family farmers around the world have been driven to destitution and suicide, and for the same reasons. Between 1993 and 1997 the number of mid-sized farms in the US dropped by 74,440(11), and farmers are now receiving below the average cost of production for their produce(12). The farming population in France and Germany fell by 50% since 1978(13). In the UK, 20 000 farming jobs were lost in the past year alone, and the Prime Minister has announced a £200m aid package(14). Four corporations control 85% of the world trade in cereals at the end of 1999(15). Mergers and acquisitions are continuing.

5. The new patents on seeds intensify corporate monopoly by preventing farmers from saving and replanting seeds, which is what most farmers still do in the Third World. In order to protect their patents, corporations are continuing to develop terminator technologies that genetic engineer harvested seeds not to germinate, despite worldwide opposition from farmers and civil society at large(16).

6. Christian Aid, a major charity working with the Third World, concluded that GM crops will cause unemployment,

exacerbate Third World debt, threaten sustainable farming systems and damage the environment. It predicts famine for the poorest countries(17). African Governments condemned Monsanto's claim that GMOs are needed to feed the hungry of the world: "We..strongly object that the image of the poor and hungry from our countries is being used by giant multinational corporations to push a technology that is neither safe, environmentally friendly, nor economically beneficial to us... we believe it will destroy the diversity, the local knowledge and the sustainable agricultural systems that our farmers have developed for millennia and ... undermine our capacity to feed ourselves.(18)" A message from the Peasant movement of the Philippines to the Organization for Economic Cooperation and Development (OECD) of the industrialized countries stated, "The entry of GMOs will certainly intensify landlessness, hunger and injustice.(19)"

7. A coalition of family farming groups in the US have issued a comprehensive list of demands, including ban on ownership of all life-forms; suspension of sales, environmental releases and further approvals of all GM crops and products pending an independent, comprehensive assessment of the social, environmental, health and economic impacts; and for corporations to be made liable for all damages arising from GM crops and products to livestock, human beings and the environment(20). They also demand a moratorium on all corporate mergers and acquisitions, on farm closures, and an end to policies that serve big agribusiness interests at the expense of family farmers, taxpayers and the environment(21). They have mounted a lawsuit against Monsanto and nine other corporations for monopolistic practices and for foisting GM crops on farmers without adequate safety and environmental impact assessments(22).

8. Some of the hazards of GM crops are openly acknowledged by the UK and US Governments. UK Ministry of Agriculture, Fisheries and Food (MAFF) has admitted that the transfer of GM crops and pollen beyond the planted fields is unavoidable(23), and this has already resulted in herbicide-tolerant weeds(24). An interim report on UK Government-sponsored field trials confirmed hybridisation between adjacent plots of different herbicide tolerant GM oilseed rape varieties, which gave rise to hybrids tolerant to multiple herbicides. In addition, GM oilseed rape and their hybrids were found as volunteers in subsequent wheat and barley crops, which had to be controlled by standard herbicides(25). Bt-resistant insect pests have evolved in response to the continuous presence of the toxins in GM plants throughout the growing season, and the US Environment Protection Agency is recommending farmers to plant up to 40% non-GM crops in order to create refugia for non-resistant insect pests(26).

9. The threats to biodiversity from major GM crops already commercialized are becoming increasingly clear. The broad-spectrum herbicides used with herbicide-tolerant GM crops decimate wild plant species indiscriminately, they are also toxic to animals. Glufosinate causes birth defects in mammals(27), and glyphosate is linked to non-Hodgkin lymphoma(28). GM crops with bt-toxins kill beneficial insects such as bees(29) and lacewings(30), and pollen from bt-corn is found to be lethal to monarch butterflies(31) as well as swallowtails(32). Bt-toxin is exuded from roots of bt-plants in the rhizosphere, where it rapidly binds to soil particles and become protected from degradation. As the toxin is present in an activated, non-selective form, both target and non-target species in the soil will be affected(33), with knock on effects on species above ground.

10. Products resulting from genetically modified organisms can also be hazardous. For example, a batch of tryptophan produced by GM microorganisms was associated with at least 37 deaths and 1500 serious illnesses(34). Genetically modified Bovine Growth Hormone, injected into cows in order to increase milk yield, not only causes excessive suffering and illnesses for the cows but increases IGF-1 in the milk, which is linked to breast and prostate cancers in humans(35). It is vital for the public to be protected from all GM products, and not only those containing transgenic DNA or protein. That is because the process of genetic modification itself, at least in the form currently practised, is inherently unsafe.

11. Secret memoranda of US Food and Drug Administration revealed that it ignored the warnings of its own scientists that genetic engineering is a new departure and introduces new risks. Furthermore, the first GM crop to be commercialized - the Flavr Savr tomato - did not pass the required toxicological tests(36). Since then, no comprehensive scientific safety testing had been done until Dr. Arpad Pusztai and his collaborators in the UK raised serious concerns over the safety of the GM potatoes they were testing. They conclude that a significant part of the toxic effect may be due to the "[gene] construct or the genetic transformation (or both)" used in making the GM plants(37).

12. The safety of GM foods was openly disputed by Professor Bevan Moseley, molecular geneticist and current Chair of the Working Group on Novel Foods in the European Union's Scientific Committee on Food(38). He drew attention to unforseen effects inherent to the technology, emphasizing that the next generation of GM foods - the so-called 'neutraceuticals' or 'functional foods', such as vitamin A 'enriched' rice - will pose even greater health risks because of the increased complexity of the gene constructs.

13. Genetic engineering introduces new genes and new combinations of genetic material constructed in the laboratory into crops, livestock and microorganisms(39). The artificial constructs are derived from the genetic material of pathogenic viruses and other genetic parasites, as well as bacteria and other organisms, and include genes coding for antibiotic resistance. The constructs are designed to break down species barriers and to overcome mechanisms that prevent foreign genetic material from inserting into genomes. Most of them have never existed in nature in the course of billions of years of evolution.

14. These constructs are introduced into cells by invasive methods that lead to random insertion of the foreign genes into the genomes (the totality of all the genetic material of a cell or organism). This gives rise to unpredictable, random effects, including gross abnormalities in animals and unexpected toxins and allergens in food crops.

15. One construct common to practically all GM crops already commercialized or undergoing field trials involves a gene-switch (promoter) from the cauliflower mosaic virus (CaMV) spliced next to the foreign gene (transgene) to make it over-express continuously(40). This CaMV promoter is active in all plants, in yeast, algae and E. coli. We recently discovered that it is even active in amphibian egg(41) and human cell extract(42). It has a modular structure, and is interchangeable, in part, or in whole with promoters of other viruses to give infectious viruses. It also has a 'recombination hotspot' where it is prone to break and join up with other genetic material(43).

16. For these and other reasons, transgenic DNA - the totality of artificial constructs transferred into the GMO - may be more unstable and prone to transfer again to unrelated species; potentially to all species interacting with the GMO(44).

17. The instability of transgenic DNA in GM plants is well-known(45). GM genes are often silenced, but loss of part or all of the transgenic DNA also occurs, even during later generations of propagation(46). We are aware of no published evidence for the long term stability of GM inserts in terms of structure or location in the plant genome in any of the GM lines already commercialized or undergoing field trials.

18. The potential hazards of horizontal transfer of GM genes include the spread of antibiotic resistance genes to pathogens, the generation of new viruses and bacteria that cause disease and mutations due to the random insertion of foreign DNA, some of which may lead to cancer in mammalian cells(47). The ability of the CaMV promoter to function in all species including human beings is particularly relevant to the potential hazards of horizontal gene transfer.

19. The possibility for naked or free DNA to be taken up by mammalian cells is explicitly mentioned in the US Food and Drug Administration (FDA) draft guidance to industry on antibiotic resistance marker genes(48). In commenting on the FDA's document, the UK MAFF pointed out that transgenic DNA may be transferred not just by ingestion, but by contact with plant dust and air-borne pollen during farm work and food processing(49). This warning is all the more significant with the recent report from Jena University in Germany that field experiments indicated GM genes may have transferred via GM pollen to the bacteria and yeasts in the gut of bee larvae(50).

20. Plant DNA is not readily degraded during most commercial food processing(51). Procedures such as grinding and milling left grain DNA largely intact, as did heat-treatment at 90deg.C. Plants placed in silage showed little degradation of DNA, and a special UK MAFF report advises against using GM plants or plant waste in animal feed.

21. The human mouth contains bacteria that have been shown to take up and express naked DNA containing antibiotic resistance genes, and similar transformable bacteria are present in the respiratory tracts(52).

22. Antibiotic resistance marker genes from GM plants have been found to transfer horizontally to soil bacteria and fungi in the laboratory(53). Field monitoring revealed that GM sugar beet DNA persisted in the soil for up to two years after the GM crop was planted. And there is evidence suggesting that parts of the transgenic DNA have transferred horizontally to bacteria in the soil(54).

23. Recent research in gene therapy and nucleic acid (both DNA and RNA) vaccines leaves little doubt that naked/free nucleic acids can be taken up, and in some cases, incorporated into the genome of all mammalian cells including those of human beings. Adverse effects already observed include acute toxic shock, delayed immunological reactions and autoimmune reactions(55).

24. The British Medical Association, in their interim report (published May, 1999), called for an indefinite moratorium on the releases of GMOs pending further research on new allergies, the spread of antibiotic resistance genes and the effects of transgenic DNA.

25. In the Cartegena Biosafety Protocol successfully negotiated in Montreal in January, 2000, more than 130 governments have agreed to implement the precautionary principle, and to ensure that biosafety legislations at the national and international levels take precedence over trade and financial agreements at the WTO. Similarly, delegates to the Codex Alimentarius Commission Conference in Chiba Japan, March 2000, have agreed to prepare stringent regulatory procedures for GM foods that include pre-market evaluation, long-term monitoring for health impacts, tests for genetic stability, toxins, allergens and other unintended effects(56). The Cartegena Biosafety Protocol has now been signed by 68 Governments in Nairobi in May, 2000.

26. We urge all Governments to take proper account of the now substantial scientific evidence of actual and suspected hazards arising from GM technology and many of its products, and to impose an immediate moratorium on further environmental releases, including open field trials, in accordance with the precautionary principle as well as sound science.

27. Successive studies have documented the productivity and sustainability of family farming in the Third World as

well as in the North(57). Evidence from both North and South indicates that small farms are more productive, more efficient and contribute more to economic development than large farms. Small farmers also tend to make better stewards of natural resources, conserving biodiversity and safeguarding the sustainability of agricultural production(58). Cuba responded to the economic crisis precipitated by the break up of the Soviet Bloc in 1989 by converting from conventional large scale, high input monoculture to small organic and semi-organic farming, thereby doubling food production with half the previous input(59).

28. Agroecological approaches hold great promise for sustainable agriculture in developing countries, in combining local farming knowledge and techniques adjusted to local conditions with contemporary western scientific knowledge(60). The yields have doubled and tripled and are still increasing. An estimated 12.5 million hectares worldwide are already successfully farmed in this way(61). It is environmentally sound and affordable for small farmers. It recovers farming land marginalized by conventional intensive agriculture. It offers the only practical way of restoring agricultural land degraded by conventional agronomic practices. Most of all, it empowers small family farmers to combat poverty and hunger.

29. We urge all Governments to reject GM crops on grounds that they are both hazardous and contrary to ecologically sustainable use of resources. Instead they should support research and development of sustainable agricultural methods that can truly benefit family farmers the world over.

[sort by surname][sort by country]

signed by

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141 **Dr. John C. Worketin** Retired computer scientist Ontario Canada
142 **Werner Zimmermann** interested informed and concerned citizen Canada
143 **Tea Garcia-Huidobro M.Sc** Biochemistry (B.Sc) and Environmental Technology (M.Sc) Chile
144 **Diana Medel** Studies on Anthropol Soc Soc Psy Member Anthroposophic Society Volunteer for Children sRights Garden s Constr Inv Med Plants Food Environm Stud ONG to supervise Children s Rights Chile
145 **JUAN DU B.Eng** civil Engineering Warwick U K China
146 **Dr. Ye Hua** over 20 000 pieces hand painted oil painting and picture frames in stock for sale at lowest prices http www art98 com China
147 **Dr. Alexander Jablanczy** General Practitioner Doctor's Building Saulte Ste. Marie China
148 **Dr. Jesse LiLing M.D** Bioinformation Tsinghua University China
149 **William Bingbin Lui Ph.D student** I strongly support such an effort I think both National and International legal regimes should accept this open letter Law School Fudan Universitry Shanghai China
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1. See World Scientists' Statement, Institute of Science in Society website <www.i-sis.org.uk>
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Pollen- and Seed-Mediated Transgene Flow in Commercial Cotton Seed Production Fields

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Abstract

Background: Characterizing the spatial patterns of gene flow from transgenic crops is challenging, making it difficult to design containment strategies for markets that regulate the adventitious presence of transgenes. Insecticidal *Bacillus thuringiensis* (Bt) cotton is planted on millions of hectares annually and is a potential source of transgene flow.

Methodology/Principal Findings: Here we monitored 15 non-Bt cotton (*Gossypium hirsutum*, L.) seed production fields (some transgenic for herbicide resistance, some not) for gene flow of the Bt cotton *cry1Ac* transgene. We investigated seed-mediated gene flow, which yields adventitious Bt cotton plants, and pollen-mediated gene flow, which generates outcrossed seeds. A spatially-explicit statistical analysis was used to quantify the effects of nearby Bt and non-Bt cotton fields at various spatial scales, along with the effects of pollinator abundance and adventitious Bt plants in fields, on pollen-mediated gene flow. Adventitious Bt cotton plants, resulting from seed bags and planting error, comprised over 15% of plants sampled from the edges of three seed production fields. In contrast, pollen-mediated gene flow affected less than 1% of the seed sampled from field edges. Variation in outcrossing was better explained by the area of Bt cotton fields within 750 m of the seed production fields than by the area of Bt cotton within larger or smaller spatial scales. Variation in outcrossing was also positively associated with the abundance of honey bees.

Conclusions/Significance: A comparison of statistical methods showed that our spatially-explicit analysis was more powerful for understanding the effects of surrounding fields than customary models based on distance. Given the low rates of pollen-mediated gene flow observed in this study, we conclude that careful planting and screening of seeds could be more important than field spacing for limiting gene flow.

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Introduction

Gene flow between sexually compatible crops typically decreases as the distance between crops increases. Thus, growers who intend to minimize gene flow from surrounding crop varieties commonly do so by increasing the spacing between fields [1]. Nevertheless, transgene flow (i.e., gene flow of a genetically engineered trait) into commercial agricultural seed lots is documented in maize, canola, soybean, and cotton [2–5]. As transgenic plants, grown by 14 million farmers in 25 countries [6], are a dominant landscape feature in many regions, some transgene flow is inevitable [7,8]. However, substantial transgene flow could threaten the intellectual property rights of biotechnology companies, markets for non-transgenic products, and resistance management strategies for insects and weeds [4,9–12].

Transgene flow can occur via pollen-mediated gene flow or seed-mediated gene flow [11]. Pollen-mediated transgene flow (''outcrossing'') occurs when plants without a particular transgene are cross-pollinated by plants with the transgene. If the resulting seeds are planted, ''adventitious presence'' occurs in fields the following year. In contrast, seed-mediated transgene flow results from volunteer transgenic plants emerging in fields, adventitious presence in the planted seed, or human error during planting, harvesting, or seed processing. Seed-mediated gene flow can enhance pollen-mediated gene flow when ''adventitious plants'' arising from seed-mediated gene flow cross-pollinate surrounding plants [3,5,13,14]. For cultivated cotton (*Gossypium hirsutum*, L.), which is the focus of our study, vegetative dispersal does not occur in the field [15] and, therefore, is not considered here.

Empirical field data on transgene flow are critical for modelers and decision makers who wish to develop containment strategies [1]. Most empirical studies have been relatively simple and focused on pollen-mediated gene flow [1]. While simulation models have explored the simultaneous roles of pollen vectors, field spacing, and adventitious plants on pollen-mediated gene flow rates [16,17], statistical analyses of empirical data have not simulta-

neously quantified these effects. Several empirical studies have statistically described the decline in transgene flow with distance from the nearest source of transgenic plants [e.g., 13, 18, 19], but this approach can be imprecise in complex agricultural landscapes with many sources of transgenic plants. Thus, we saw a need for a spatially explicit model that would account for the area and distance of all relevant neighboring fields, along with the effects of pollen vectors and adventitious plants, to evaluate the causes of pollen-mediated gene flow in commercial fields.

Relatively little gene flow research focuses on cotton, although it is the third most abundant genetically engineered crop [6]. This is likely because it is a self-pollinating crop with low outcrossing rates. While the ability of transgenic *Bacillus thuringiensis* (Bt) cultivars of *G. hirsutum* to cross-pollinate non-Bt *G. hirsutum* is well-documented [15,20–22], pollen-mediated transgene flow rates in cotton rarely exceed 1% of seeds at a distance of 10 meters into a field [15,20–23]. Nevertheless, in 2004, we found 7.5–8% adventitious presence of Bt cotton in non-Bt cotton experimental plots in Arizona, USA, likely resulting from adventitious presence in the planted seed [5]. In subsequent testing of commercial non-Bt cotton seed bags, three out of eleven bags contained 1% Bt seed, as indicated by the presence of the Bt protein Cry1Ac [5]. The source of this gene flow was unknown [5].

Outcrossing in cotton is mediated by bees and not by wind [23], which presents a challenge for modelers, because the precise relationship between pollinators and gene flow is difficult to quantify [1]. Two studies of transgene flow in cotton each reported that a location with abundant bees had higher outcrossing than a location with few bees [22,23]. However, while knowledge of pollinator effects is crucial for modeling gene flow in insect-pollinated crops [24], other field studies have not precisely quantified the effect of pollinator density on transgene flow rates in cotton or any other crop.

Here, we evaluated the relative importance of pollen- and seed-mediated gene flow in the spread of the *cry1Ac* transgene into non-Bt cotton seed production fields, and developed a spatially-explicit statistical model for characterizing gene flow from multiple fields. We used geographic information system (GIS) and multiple logistic regression tools to simultaneously test the hypotheses that pollen-mediated gene flow would: 1) increase as the area of nearby Bt cotton fields increased, 2) decrease as nearby non-Bt cotton increased [25], 3) increase as the abundance of pollinating insects increased, and 4) increase as the abundance of adventitious Bt cotton plants increased. We also evaluated the spatial scale of pollen-mediated gene flow, the extent of seed-mediated gene flow from volunteer plants, and adventitious presence in the planted seed.

Methods

Transgene flow from Bt cotton to non-Bt cotton was monitored in approximately 130 ha of non-Bt cotton seed production fields in Arizona, USA in 2007. Such fields are grown by farmers under contract with seed companies and are used to produce both lint and seed. We selected three farms in western, central, and eastern Arizona, respectively, that we believed to be representative of cotton seed production fields in Arizona. From these farms, 15 non-Bt cotton seed production fields, which ranged from 2.5 to 16 ha, were selected based on, 1) availability of subsampled seed from the planted seed lot, 2) receiving news of the field before the rows were cultivated for weed management, 3) accessibility, and 4) maximizing the distance between monitored fields (no adjacent fields were selected). Although we used the Bt protein Cry1Ac as a marker for gene flow from Bt cotton, we note that some cotton grown in Arizona produces two Bt proteins: Cry1Ac and Cry2Ab. Five non-Bt cotton varieties were represented in the monitored fields, of which four varieties were transgenic for glyphosate resistance.

Examining Sources of Seed-Mediated Gene Flow

We tested seed from the six seed lots used in planting the 15 monitored fields for Cry1Ac. Seed samples were provided by growers and were collected from seed bags or recently filled hoppers on the planting equipment. When possible, we collected multiple seed samples from a seed lot for archiving. From each seed lot, 200 seeds were tested with a lateral flow immunoassay (Cry1Ab/Ac ImmunoStrips, Agdia Inc., Elkhart, IN). Each seed was halved, with one half of the kernel tested in a pool and the other half archived. Pools of 25 seed halves were tested together, with pools of 24 non-Bt seed halves plus one Bt seed half serving as positive controls, and buffer as the negative control. We followed the manufacturer's protocol, but increased extraction time from 30 s to 2 h to yield clearer test results [5]. All controls (20 positive, 20 negative) produced expected results. For pools testing positive, archived seed halves were tested with ImmunoStrips following the manufacturer's guidelines to quantify the number of Bt seeds in the pool [5]. The proportion of adventitious presence of the *cry1Ac* transgene in each seed lot was estimated as the number of Cry1Ac positive seeds divided by the total number of seeds tested.

To quantify volunteer plants emerging from the soil seed bank, we walked a minimum of four transects through each field, inspecting a minimum of eight rows soon after plants emerged but before rows were cultivated to manage weeds. We noted and sampled cotton plants outside of rows and residual cotton lint with seeds in the soil.

Assessing Factors That Enhance Pollen-Mediated Gene Flow

We monitored pollinator activity in fields every two weeks throughout peak flowering with visual surveys. Fields were monitored two to five times, depending on their flowering period and accessibility. Fields were inaccessible during flood irrigation, and some fields were frequently flooded. For visual monitoring, an entomologist walked a consistent pace (~0.5 m/s) along the centermost row of a field and both edge rows, counting the number of open white flowers and the number of pollinating insects (i.e., insects moving among flowers and foraging inside flowers) [26]. Thus, approximately 5,000–13,000 plants, depending on field size, were surveyed during each monitoring, which lasted 20 min. to 1 hr. For consistency, the same entomologist performed all monitoring. Honey bees (*Apis mellifera* L.) were identified to species while other pollinators were recorded and, when possible, collected for future identification. Nearly all pollinating insects were bees, with moths and wasps seen on rare occasion. Bumble bees (*Bombus spp.*) were not seen. For each field, the average number of honey bees and native bees per flower (i.e., bee densities) were separately calculated by dividing the total number of honey bees or native bees by the total number of flowers observed across monitoring dates [26].

Maps of all Arizona cotton fields in 2007, including identities of non-Bt and Bt cotton fields, were obtained from the Arizona Cotton Research and Protection Council [27]. Using ArcView GIS Version 3.1 [28], we drew twelve rings around the edge of each seed production field, with the first ring 250 m from the field edge, and each successive ring increasing in distance by 250 m (Fig. 1). The area of Bt and non-Bt cotton between the field edge and each ring (m^2) was calculated with ArcView [29]. We

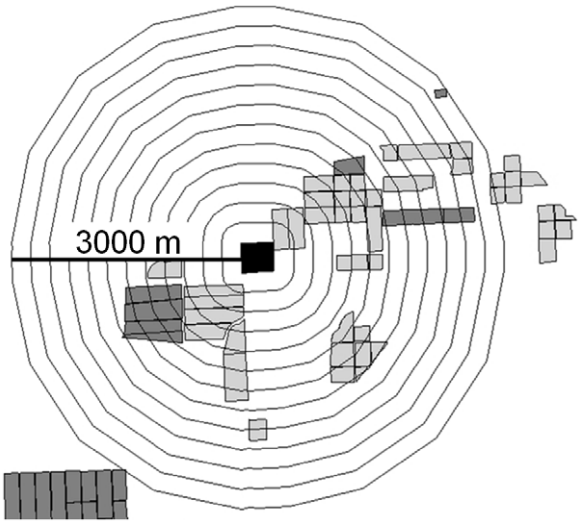

Figure 1. Diagram of rings drawn around a hypothetical cotton field. The first ring is 250 m from the field edge, and each subsequent ring increases in radius by 250 m. The area of non-Bt and Bt cotton was measured at each increasing scale. Light and dark gray represent non-Bt and Bt cotton, respectively, and the black rectangle represents a monitored non-Bt cotton field. For actual monitored fields, some rings overlapped those of nearby monitored fields.
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observed substantial overlap in the flowering periods of monitored fields and neighboring Bt and non-Bt cotton fields.

Plant Sampling and Analysis

While monitoring of pollinators and volunteer plants was performed in both edge and middle rows, we focused on sampling edge plants at the time of harvest. Pollen-mediated gene flow rates in cotton tend to be low and therefore are easiest to detect at the field edge, where rates tend to be highest [22]. Therefore, focusing on field edges allowed us to draw connections between explanatory variables and outcrossing rates by testing hundreds of seeds per field from the edges, rather than thousands of seeds from the center.

For each field, shortly before harvest, we sampled mature cotton bolls from each of 100 plants (one boll per plant) from the four outer edges of the field (25 plants per edge). We equally sampled bolls from low, middle and high positions on the plants [20]. We sampled plants from the centermost 25 m of each field edge, as defined with GPS (eTrex Legend, Garmin). We also sampled 25 plants from corresponding interior sections 20 m into the field from each edge, but bolls from some of the interior sections were not analyzed (see below).

To assess pollen- and seed-mediated gene flow, bolls were tested for Cry1Ac with ImmunoStrips. We first tested bolls from field edges. Then, for each field from which outcrossing was identified at the edge, we randomly selected one edge with outcrossing and tested bolls from its corresponding interior sample. This method allowed us to investigate outcrossing levels further into the fields. Although we only collected full-sized bolls, some bolls from edge samples did not contain mature, testable seeds, decreasing the number of replicates (Table 1). In all, we analyzed samples from 1,211 plants (12,908 seeds from 1,211 bolls) from edges and, from fields with detected outcrossing, 240 plants (2,400 seeds from 240 bolls) from the interiors (Table 1).

From each tested boll, we first tested 10 subsampled seeds as a pool and followed up with individual seed tests for Cry1Ac positive pools, as described above for seed bag samples. For bolls with <10 mature seeds, all seeds were tested in the pool. We also tested tissue from the pericarp (i.e., fruit wall) of bolls with Bt seeds to differentiate between adventitious Bt plants and non-Bt plants

Table 1. Pollen-mediated gene flow of the *cry1Ac* transgene in non-Bt cotton fields, sample sizes, and field attributes.

Field	Plants (n)[1]			Distance to nearest Bt cotton field (m)	HB/100 flowers[2]	Pollen-mediated gene flow (% of seeds)		
	Total Edge	Paired Edge	20 m			Total Edge	Paired Edge	20 m
A	77	15	24	727	0.15	0.63	3.1	0
B	78	15	24	245	0.25	0.17	1.0	0
C	87	24	24	5	0.033	0.48	0.83	0
D	78	---	---	11	0	0	---	---
E	78	15	24	33	0	0.13	0.67	0
F	96	24	24	8	0.014	0.42	1.7	0
G	78	15	24	578	0.45	0.51	1.3	0
H	78	---	---	951	0.28	0	---	---
I	78	24	24	835	2.4	0.13	0.42	2.6
J	67	24	24	666	1.5	0.15	0.42	0
K	87	24	24	12	0.8	0.71	0.87	1.7
L	78	24	24	943	2.5	0.13	0.44	0.83
M	77	---	---	1997	2.2	0	---	---
N	87	---	---	9	0	0	---	---
O	87	---	---	9	0	0	---	---

[1]Number of tested plants, including the total number of edge plants, the number of edge plants included in the paired analysis (where applicable), and the number of plants collected 20 m in from the field edge for paired analysis (where applicable).
[2]Honey bee (HB) density from visual monitoring (honey bees/100 flowers).
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outcrossed by Bt pollen [5]. Bt-outcrossing (pollen-mediated gene flow) was identified by bolls with Bt toxin detected in some of the seeds but not in the maternal pericarp tissue. However, adventitious Bt plants (seed-mediated gene flow) were identified by detectable Bt toxin in both seeds and pericarp tissues. Adventitious Bt plants were further sorted by whether they contained only Bt seeds or both Bt and non-Bt seeds. Bt plants producing both seed types are hemizygous and average 75% seeds with the Bt trait when they self-pollinate [30]. Calculating the relative proportions of hemizygous versus homozygous plants yields insight into the source of adventitious plants, as hemizygous plants result from cross-pollination events between Bt and non-Bt cotton in previous generations [5].

Controls were run simultaneously with ImmunoStrips tests. For seed pool tests, we used 10 pooled non-Bt cotton seeds as negative controls, and one Bt cotton seed plus nine non-Bt cotton seeds as positive controls. Seventy pairs of controls were run, and all produced expected results. For individual seed tests, 20 control pairs of individual Bt and non-Bt cotton seed halves were tested and produced expected results. For pericarp testing, pericarp samples from Bt and non-Bt cotton bolls were used as controls. Out of seven control pairs, one negative control produced a weak false positive result. As expected, all samples with positive pericarp tests contained $\geq$60% Bt seeds, while samples with negative pericarps had $\leq$20% Bt seeds, confirming the test's utility for differentiating between pollen- and seed-mediated gene flow [5].

Statistics

We used multiple logistic regression followed by likelihood ratio tests to assess the effects of the explanatory variables on the odds of pollen-mediated gene flow. To do this, we used the nominal logistic regression platform and the generalized linear model platform in JMP 8.0 (SAS Institute [31]). Both platforms produced the same results, but the nominal logistic regression platform provided odds ratios and their confidence intervals, while the generalized linear model platform facilitated tests for overdispersion. To avoid bias, the procedure for building our statistical model was determined in advance, including the experimental unit, response variable, statistical test, and criteria for excluding explanatory variables from the final model.

Because the same bee visit could result in cross-pollination of multiple ovules in a cotton flower, we considered individual bolls, rather than individual seeds, as the experimental unit in statistical analyses. This is identical to an analysis with individual plants as the experimental unit, as only one boll was collected from each sampled plant. The response variable was a binomial count of the number of Bt-outcrossed and non-outcrossed seeds in individual bolls from non-Bt cotton plants at the edge of monitored fields. Explanatory variables included the total area of Bt cotton and the total area of non-Bt cotton in a designated ring around each monitored field, pollinator density in the monitored field (honey bees or native bees per flower), and the proportion adventitious Bt cotton plants at the edge of the monitored field. Transformations of explanatory variables were performed, as needed, to meet assumptions of linearity and homogeneity of the residuals. A summary of the explanatory variables and their transformations is included in Table 2.

The analysis was performed separately for each spatial scale (Fig. 1), with the area of nearby Bt and non-Bt cotton fields varying among spatial scales, while bee densities and the proportion of adventitious Bt plants remained constant. We also considered the interaction between adventitious Bt plants and the area of Bt cotton at each spatial scale, as we suspected that adventitious Bt plants would diminish the association between nearby Bt cotton fields and outcrossing, based on findings from our 2004 field study [5].

The uncertainty (U) coefficient of determination (R^2) is the proportion of variation (uncertainty) in the dataset that is attributable to the logistic regression model. This parameter is equivalent to the R^2 used in linear regression, but tends to be much lower in logistic regression because it depends on the negative sum of the logs of observed probabilities [31]. As we increased the spatial scale of analysis (Fig. 1), we expected R^2 to increase if the added area helped to explain outcrossing, but to decrease once the scale exceeded the distance to which outcrossing occurred. Thus, we plotted R^2 for each spatial scale and used the scale with a maximum R^2 in our final analysis [29]. Explanatory variables for which $P>0.05$ at all spatial scales of the analysis were excluded from the final model.

Previous studies modeled gene flow as a function of distance from the nearest transgenic source field. To compare this method with our spatially-explicit approach, we performed a logistic regression analysis where the shortest distance from each monitored field to the nearest Bt cotton field (log transformed) was substituted for the area of neighboring Bt cotton. For both the distance model and spatially-explicit model, deviance goodness-of-fit tests and overdispersion parameters (values $\neq$1 conflict with the assumption of binomial distribution) were used to determine whether the sample data followed a binomial distribution, and corrections for overdispersion were applied where needed [31].

Finally, we compared outcrossing in samples from the edge of fields versus the interior of fields (20 m inside of fields) to test the hypothesis that outcrossing declines with distance into a field. To do this, for each field we subtracted the proportion of sampled

Table 2. Summary of explanatory variables included in the full logistic regression analysis.

Variable	Transformation	Constant across scales of analysis?
1. Honey bee density (bees per flower)	arcsine$\sqrt{x}$	Yes. Measurement was from the monitored field.
2. Native bee density (bees per flower)	arcsine$\sqrt{x}$	Yes. Measurement was from the monitored field.
3. Area of Bt cotton in neighboring fields (ha)	log (x+1)	No. Variable calculated separately for each spatial scale of analysis.
4. Area of non-Bt cotton in neighboring fields (ha)	log (x+1)	No. Variable calculated separately for each spatial scale of analysis.
5. Proportion of plants that were adventitious Bt cotton plants	arcsine$\sqrt{x}$	Yes. Measurement was from the monitored field.
6. Interaction between variables 3 and 5	N/A	No. Contained variable 3, which changed with scale.

Variables that were not significant ($\alpha>0.05$) at any of the spatial scales in the model with all 15 fields were excluded from further analyses.
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bolls from non-Bt cotton plants that contained Bt-outcrossed seeds in the interior samples from the proportion in their paired edge samples. We then used a one-tailed, paired *t*-test to determine whether this difference was greater than zero. All of the above statistics were performed with JMP 8.0 [31].

To determine the sampling power of our study, we ran a resampling program where 1000 samples of the sizes used in our study were drawn from a population with a hypothesized rate of Bt seeds or plants. Averaged across samples, adventitious presence was always equal to the rate specified in simulations, but some samples did not detect Bt seeds or plants. From these simulated samples, we determined the proportion from which at least one positive seed or plant was detected. In our testing of seed lots (n = 200 seeds/lot), we had an 86.0% chance of detecting adventitious presence in a seed lot if the gene flow rate was 1%, and a 98.8% chance of detecting it if the rate was 2%. Our rate of detecting Bt-outcrossed seeds in any given cotton field (n = ~800 seeds/field) was 87.7% if the true outcrossing rate was 0.25%, and 98.1% if the outcrossing rate was 0.5%. The probability of detecting adventitious Bt cotton plants in an individual field at our sample size of ~80 plants per field was 96.5%, 86.8%, or 62.3% if the true proportion of adventitious Bt cotton plants was 3.75% (3/80), 2.5% (2/80), or 1.25% (1/80), respectively.

Results

Two of the six seed lots used to plant the monitored non-Bt cotton seed production fields contained detectable levels of Bt cotton seed, as indicated by presence of the Cry1Ac protein. Seed Lot I contained 20% Bt seed, while Seed Lot II contained 0.5% Bt seed (Table 3). After finding the seed bag with 20% Bt seed, we tested 25 seeds from a second seed bag from the same seed lot and found 28% Bt seed. Seed Lot I was used to plant two of the 15 monitored fields, from which 17% (field *A*) and 23% (field *B*) of plants sampled from field edges were adventitious Bt plants (Table 3). Thus, adventitious presence of the *cry1Ac* transgene was consistent throughout this seed lot based on two estimates from seed bags (mean = 24%) and two estimates from tested cotton plants (mean = 20%). Plotting the distribution of adventitious Bt plants across fields revealed fields *A* and *B* to be outlier data points. Therefore, logistic regression analyses for outcrossing were performed with and without these fields.

A high estimated rate of adventitious presence in a third field was attributed to planting error (field *C*, Table 3). All plants tested from one edge were adventitious Bt plants (n = 24), yet no plants from the other three edges contained Cry1Ac (n = 63). We tested one plant from the corresponding interior sample to determine the extent of the planting mistake. It was negative, indicating that fewer than 20 rows were affected. Because adventitious presence was not uniform throughout field *C*, the misplanted edge was considered to be part of an adjacent Bt cotton field for statistical analyses. Adventitious Bt plants were identified in 10 of the 15 fields, with a median rate of 1% of plants sampled from field edges (Table 3).

Pollen-mediated gene flow from Bt cotton was rare (Table 1). On average, only 0.23% of seeds from non-Bt cotton plants at field edges contained Cry1Ac (n = 15 fields, 95% confidence interval (CI) = 0.092–0.37%). At any scale of analysis (Fig. 1), the area of neighboring non-Bt cotton and the density of native bees in monitored fields were not significantly associated with the odds of Bt-outcrossing of non-Bt cotton plants ($P>0.05$), after accounting for the effects of the other explanatory variables. Thus, these factors were excluded from the statistical model.

Our final model of pollen-mediated gene flow included the density of honey bees in monitored fields, the proportion of adventitious Bt cotton plants in monitored fields, the area of Bt cotton fields surrounding the monitored fields (using various spatial scales of analysis, Fig. 1), and the interaction between these

Table 3. Seed-mediated gene flow of the *cry1Ac* transgene in monitored non-Bt cotton fields.

Field	Seed lot	Adventitious presence in planted seed (%)	Adventitious plants[1] (%)		Hemizygous[2] (%)	Source[3]
			Edge	20 m		
A	I	20	17	17	5.9	Seed bag
B	I	20	23	25	4.2	Seed bag
C	II	0.5	28	0	13	Planting error
D	II	0.5	0	---	---	---
E	II	0.5	0	0	---	---
F	II	0.5	1.0	0	100	Seed bag
G	III	0	0	0	---	---
H	III	0	0	---	---	---
I	IV	0	0	4.2	100	Unknown
J	IV	0	0	4.2	100	Unknown
K	IV	0	2.3	0	100	Unknown
L	IV	0	1.3	0	0	Unknown
M	V	0	0	---	---	---
N	VI	0	1.1	---	0	Unknown
O	VI	0	2.3	---	0	Unknown

[1]Percentage of plants that were adventitious Bt cotton plants in samples taken from the field edge or 20 m in from a field edge, if applicable.
[2]Percentage of adventitious Bt cotton plants that were hemizygous for the Bt trait.
[3]Putative source of seed-mediated gene flow.
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last two factors. The uncertainty coefficient of determination (R^2) peaked at a scale of 750 m from the field edge for models with and without fields *A* and *B* (Fig. 2). However, R^2 was lower when scales beyond 750 m (1000–3000 m) were considered, suggesting that Bt cotton at distances of more than 750 m from the field edge did not affect outcrossing (Fig. 2). Therefore, we assessed factors affecting outcrossing at the 750 m scale.

At the 750 m scale, the area of Bt cotton surrounding a seed production field and the density of foraging honey bees were positively associated with the odds of Bt-outcrossing of non-Bt cotton plants for models with or without fields *A* and *B* (Table 4, Table 5). For the model with all 15 fields, the proportion of adventitious Bt cotton plants in the monitored fields was also positively associated with Bt-outcrossing (Table 4, Table 5), and there was a significant negative interaction between the area of nearby Bt cotton fields and adventitious Bt plants (Table 4). Thus, as the proportion of adventitious Bt plants in seed production fields increased, the effect of nearby Bt cotton fields on outcrossing rates declined. However, the contribution of adventitious Bt cotton plants was not statistically significant in the model without fields *A* and *B* (Table 4). The equation for the odds of pollen-mediated gene flow in the final model with all 15 fields was: logit(π) = −11.7+22.0(honey bee density) +0.40(area of Bt cotton within 750 m) +17.4(adventitious Bt plants) – 1.5(area of Bt cotton within 750 m)(adventitious Bt plants). The following very similar equation describes the model with 13 fields: logit(π) = −11.5+22.8(honey bee density) +0.38(area of Bt cotton within 750 m) +10.7(adventitious Bt plants) – 1.1(area of Bt cotton within 750 m)(adventitious Bt plants). See Table 2 for details on the transformations of the above explanatory variables. There was no evidence of overdispersion, as the overdispersion statistic was 1.5 and lack of fit was not significant ($\chi^2 = 15$, $P = 0.14$, and $\chi^2 = 14$, $P = 0.072$ for the models with and without fields *A* and *B*, respectively).

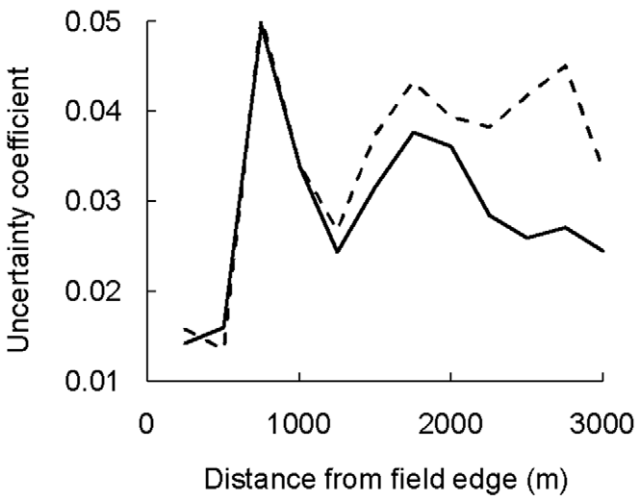

Figure 2. Uncertainty coefficient of determination (R^2) for multiple logistic regression of pollen-mediated gene flow. The area of Bt cotton at various distances from the edge of monitored non-Bt cotton fields was considered in separate analyses for each scale. Honey bee density, the proportion of plants in the monitored non-Bt cotton fields that were adventitious Bt plants, and the interaction between Bt cotton fields and adventitious Bt plants were also in the analyses. Pollen-mediated gene flow of the *cry1Ac* transgene was the response variable for the analyses. Results with fields *A* and *B* (solid line) and without fields *A* and *B* (dashed line) are shown.
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Table 4. Effect likelihood ratio tests for pollen-mediated gene flow of the *cry1Ac* transgene in monitored non-Bt cotton fields.

Explanatory variable	15 fields		13 fields	
	χ^2	Significance	χ^2	Significance
Honey bee density	10.4	$P = 0.0013$	10.4	$P = 0.0013$
Area of Bt cotton within 750 m[1]	15.5	$P < 0.0001$	13.0	$P = 0.0003$
Adventitious Bt plants (%)	11.5	$P = 0.0007$	0.66	$P = 0.42$
Interaction	10.0	$P = 0.0016$	0.96	$P = 0.33$

Significance levels (*P*-values) for each factor from models with and without fields *A* and *B* (Table 1, Table 3) are given. See Table 2 for details on the explanatory variables.
[1]Area of Bt cotton fields within 750 m of the edge of monitored non-Bt cotton fields.
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The distance between the monitored fields and their nearest neighboring Bt cotton fields (log transformed) was negatively correlated with the area of Bt cotton within 750 m of the monitored fields (log transformed) ($r = -0.86$, $P<0.0001$). For the analysis based on distance, lack of fit was significant ($\chi^2 = 28$, $P = 0.0017$, and $\chi^2 = 24$, $P = 0.0022$ for models with and without fields *A* and *B*, respectively; overdispersion = 1.9 and 2.1, respectively). Because lack of fit was significant, we corrected for overdispersion in the distance model [31]. With or without fields *A* and *B*, after correcting for overdispersion, there was no significant association between outcrossing and distance to the nearest Bt cotton field ($P \geq 0.12$), or the other factors in the model, including honey bee density ($P \geq 0.12$), adventitious Bt plants ($P \geq 0.11$), and the interaction between distance and adventitious Bt plants ($P \geq 0.28$).

In the experiment comparing paired edge and interior field samples, there was a trend for a decline in the proportion of non-Bt cotton bolls containing Cry1Ac positive seeds from the edge to the interior of fields (Table 1), but this trend was not statistically significant ($t_9 = 1.6$, one-sided $P = 0.072$). The presence of adventitious Bt plants (i.e., seed-mediated gene flow) did not differ between edge and interior samples either (paired *t*-test excluding the planting mistake in field *C*, $t_9 = 0.39$, two-sided $P = 0.71$). Similarly, honey bee densities appeared consistent across fields, with no difference between edge and middle rows (paired *t*-test, $t_{14} = 1.1$, two-sided $P = 0.29$). Honey bees comprised 88% of the observed foraging bees, while native bees were less abundant in all fields (<0.5 native bees per 100 flowers).

The seed composition of bolls revealed that ten of the 74 identified adventitious Bt plants (13.5%) were hemizygous for the *cry1Ac* transgene (see Table 3). Bolls from these plants contained, on average, 79% (95% CI = 72-86%) Bt seeds, which is not significantly different from the 3:1 ratio for hemizygous cotton plants that self-pollinate ($t_9 = 1.3$, $P = 0.24$).

We found no evidence that volunteer plants contributed to gene flow. Fewer than two plants per kilometer of monitored row (<0.01% of plants) emerged outside of planted rows, even in fields where residual cotton lint was visible. Moreover, rare plants outside of rows could have resulted from flaws in the planting machinery. As volunteer plants were an unlikely source of gene flow, we did not follow up with ImmunoStrips tests of the plants occurring outside of rows.

Table 5. Range odds ratios[1] for the effects of the explanatory variables on outcrossing.

Explanatory variable	15 fields		13 fields	
	Odds ratio[1]	Confidence interval	Odds ratio[1]	Confidence interval
Honey bee density	6.4	1.1–39	30	3.7–270
Area of Bt cotton within 750 m	9.1	1.5–65	84	6.3–2900
Adventitious Bt plants (%)	2.3	0.63–6.9	---	---

From a simplified model without the interaction term (odds ratios of interactions are difficult to interpret). Results from models with and without fields *A* and *B* are given.

[1]Range odds ratios estimate the change in the odds of an event (i.e., outcrossing) over the observed range of an explanatory variable [31]. For instance, in the field with the most honey bees, plants had 6.4-fold higher odds of outcrossing than in the field with the fewest honey bees for the 15 field model.

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Discussion

Although seed-mediated gene flow has received less attention than pollen-mediated gene flow in the literature [1], it was clearly the most prominent source of *cry1Ac* transgene flow in this study (Table 1, Table 3). Seed-mediated gene flow resulted primarily from adventitious presence in the planted seed and from planting error, although some fields with no evidence of these sources contained low percentages of adventitious Bt plants (Table 3). Some adventitious Bt plants were hemizygous for *cry1Ac* (Table 3), indicating pollen-mediated gene flow in previous generations, either of Bt pollen into non-Bt cotton plants, or of non-Bt pollen into adventitious Bt cotton plants [5]. In fields where gene flow entered via the planted seed, most adventitious Bt plants were homozygous, suggesting that seed-mediated gene flow was the original source of gene flow (Table 3, fields *A–F*).

Pollen-mediated gene flow of the *cry1Ac* transgene was also observed, but occurred at rates below 1% at field edges (Table 1). While other authors have noted the relevance of pollinator abundance, adventitious plants, and the area of surrounding crops to pollen-mediated gene flow [1,16,17], to our knowledge, this is the first empirical study to statistically describe the concurrent effects of these factors on gene flow rates. We showed that a spatially-explicit analysis based on the area of nearby crops compared favorably to the simplest distance-based analysis. Honey bees appeared to be the primary outcrossing agent in the seed production fields, which was also noted in previous cotton outcrossing studies [e.g., 22, 26, 32]. Native bees did not appear to increase outcrossing significantly, perhaps due to their low abundance. The area of Bt cotton fields within 750 m of the monitored fields best explained outcrossing rates, as the explanatory power of the model was lower at smaller or larger scales (Fig. 2). The 750 m scale of outcrossing falls within the foraging range of honey bees, which has been documented at over 3000 m [33]. We expected neighboring non-Bt cotton fields to reduce Bt-outcrossing by acting as an alternative sink for Bt pollen and as a competing pollen source, but did not observe this effect at the sample size used.

We did not detect a significant difference in outcrossing between field edges and samples taken 20 m from the edge. We note that our study design could have potentially overestimated differences in outcrossing between the edge and interior samples, because we only tested interior samples if outcrossing was already detected at the corresponding edge. However, this would not affect our conclusion that no significant difference was observed at the sample size used. Similarly, in our 2004 study conducted in non-Bt cotton plots with 7.5–8% adventitious presence of Bt plants, we observed no significant decline in outcrossing with distance from the adjacent Bt cotton plots [5]. Small-scale field trials in other regions reported dramatic decreases in Bt-outcrossing with distances of 20 m or less into non-Bt cotton buffers surrounding Bt cotton test plots [15,20,21]. Unharvested buffers of non-transgenic plants are commonly used as a sink to contain transgenic pollen [1]. Our study showed that gene flow rates did not always drop off at 20 m. However, this result does not imply that outcrossing at the edge of fields is representative of the entire field, as samples beyond 20 m from the field edge were not taken.

We expect that pollen-mediated gene flow rates would be lower in the center of fields [22]. However, edge sampling was the most efficient way to maximize detection of outcrossing in this study, as cotton is a low outcrossing crop. We assume that the significant association between our explanatory variables and pollen-mediated gene flow rates extend to whole fields, as field edges are a point of entry into the rest of the field. A more extensive survey with higher sample sizes to detect low gene flow rates in the interior of fields would be needed to demonstrate that these explanatory variables are associated with pollen-mediated gene flow rates throughout the field.

Adventitious Bt cotton plants may have acted as a source of pollen-mediated gene flow [5,13,14] (Table 4), and enhanced outcrossing levels 20 m inside fields where little outcrossing from neighboring fields was expected. There was some evidence that adventitious Bt cotton plants diminished the association between neighboring Bt cotton fields and pollen-mediated gene flow (Table 4), suggesting that the two pollen sources may compete to outcross non-Bt cotton plants. However, the contribution of adventitious Bt cotton plants and the interaction between the two Bt pollen sources were only significant when fields *A* and *B*, which had high adventitious presence throughout (Table 3), were included in the analysis. Data from more fields with intermediate to high adventitious presence (i.e., 3-28%; see Table 3) would be needed to more fully detail the contributions of adventitious Bt cotton plants to outcrossing.

Other factors, in addition to those measured in this study, may also influence gene flow patterns. For example, the extent of overlap in flowering periods and characteristics of specific crop varieties may influence the extent of cross-pollination between any two crop patches [1]. The robust statistical association between the variables in our model (Table 4) suggests that pollinator abundance and the area of surrounding Bt cotton fields are key variables that influence pollen-mediated gene flow.

In the United States, non-transgenic crops do not require separation from transgenic crops that have received government approval [23], unless they are labeled as "GE-free" or "organic" [12]. The seed examined in this study did not have these labels. Furthermore, most of the non-Bt cotton varieties included in this study were transgenic for herbicide resistance, and thus were not intended for the GE-free or organic markets. Nevertheless,

adventitious presence of the Bt trait is a concern for non-Bt cotton refuges used in pest resistance management programs in many countries [10,34,35]. Refuges are intended to increase the proportion of Bt-susceptible insects in a pest population [36]. Adventitious presence of Bt cotton in refuges could accelerate resistance by increasing the mortality of susceptible insects or shifting the dominance of resistance [10,34].

We note that the seed produced in monitored fields may not have been sold to growers. While the United States does not have strict labeling thresholds for adventitious presence of the Bt trait in seed, seed companies sometimes voluntarily reject seed lots with adventitious presence of transgenes. However, as we observed in fields planted with the seed lot containing 20% adventitious presence (Table 3), gene flow can go overlooked and persist across generations in the seed production setting.

Although one field season does not capture variability among years, it provides a detailed snapshot of the factors that contribute to transgene flow. Results from this study suggest that crop spacing can be used to limit unwanted gene flow, as Bt cotton fields >750 m from the edge of monitored fields did not appear to contribute to outcrossing. However, pollen-mediated transgene flow rates were always low in this study (i.e., <1% of seeds at the field edge), even in monitored fields that were near Bt cotton fields (Table 1). This suggests that spacing fields hundreds of meters from transgenic crops is unnecessary for cotton, even in the European Union where the labeling threshold for adventitious presence in crops is 0.9% [37]. However, this study demonstrates the potential for seed-mediated gene flow to become prominent in settings where actions are not taken to keep adventitious presence in check. The ecological patterns underlying gene flow in this study could apply to related seed production systems, particularly for other insect-pollinated transgenic crops. In settings where seed purity is desirable, seed producers and decision makers should consider 1) screening seeds to monitor adventitious presence in the seed supply, and 2) communicating the importance of segregating seed types at planting to reduce human error.

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Author Contributions

Conceived and designed the experiments: SH BT YC. Performed the experiments: SH CEK. Analyzed the data: SH CEK YC. Contributed reagents/materials/analysis tools: SH YC. Wrote the paper: SH BT YC.

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Recent long-distance transgene flow into wild populations conforms to historical patterns of gene flow in cotton (*Gossypium hirsutum*) at its centre of origin

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Abstract

Over 95% of the currently cultivated cotton was domesticated from *Gossypium hirsutum*, which originated and diversified in Mexico. Demographic and genetic studies of this species at its centre of origin and diversification are lacking, although they are critical for cotton conservation and breeding. We investigated the actual and potential distribution of wild cotton populations, as well as the contribution of historical and recent gene flow in shaping cotton genetic diversity and structure. We evaluated historical gene flow using chloroplast microsatellites and recent gene flow through the assessment of transgene presence in wild cotton populations, exploiting the fact that genetically modified cotton has been planted in the North of Mexico since 1996. Assessment of geographic structure through Bayesian spatial analysis, BAPS and Genetic Algorithm for Rule-set Production (GARP), suggests that *G. hirsutum* seems to conform to a metapopulation scheme, with eight distinct metapopulations. Despite evidence for long-distance gene flow, genetic variation among the metapopulations of *G. hirsutum* is high (He = 0.894 ± 0.01). We identified 46 different haplotypes, 78% of which are unique to a particular metapopulation, in contrast to a single haplotype detected in cotton cultivars. Recent gene flow was also detected (m = 66/270 = 0.24), with four out of eight metapopulations having transgenes. We discuss the implications of the data presented here with respect to the conservation and future breeding of cotton populations and genetic diversity at its centre of crop origin.

Keywords: *Gossypium hirsutum*, long distance gene flow, metapopulations, Mexico, transgene flow

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Introduction

The complexes of wild and cultivated varieties of crop plants at their centres of crop origin and/or diversity (hereafter, CCO) provide useful systems for addressing fundamental questions on population structure, genetics, and specifically, gene flow dynamics (e.g. maize to teosinte; Baltazar *et al.* 2005; Ellstrand *et al.* 2007; the beet family; Bartsch *et al.* 1999; Viard *et al.* 2004; Fénart *et al.* 2007; Arnaud *et al.* 2009; or *Brassica* spp. Jørgensen & Andersen 1994; Snow *et al.* 1999). In cases where genetically modified varieties have been released at the CCO, transgenes become useful markers for addressing ongoing patterns, dynamics, and pervasiveness of gene flow (maize, van Heerwaarden *et al.* 2009; *Cucurbita*, Sasu *et al.* 2009; *Sorghum*, Sahoo *et al.* 2010). At the same time, these cases become particularly relevant for assessing the

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general viability of GMO cultivation when there is potential for transgene flow into wild relatives at the CCO.

In spite of the effects of recent gene flow involving transgenes or other genetic elements, historical gene flow may still have a dramatic impact on population genetic structure (Ehrlich & Raven 1969). It may counteract the effects on effective population size of drift and inbreeding (Ebert *et al.* 2002), but may also constrain population differentiation by homogenizing the gene pool (Slatkin 1987). Gene flow estimation has historically relied on estimates of *Nm* (number of migrants per generation) and F_{st} (Fixation index; a measure of population differentiation). However, both of these parameters have been developed based on simplified and typically unrealistic population models (Whitlock & McCauley 1999; Paetkau *et al.* 2004) that assume, for example, that populations are at equilibrium (Broquet & Petit 2009).

In contrast, the use of haplotype networks and genetic covariance estimates, such as those used in Popgraph analyses, can provide information regarding the historical and spatial relationships among genotypes (Dyer 2009). For instance, historical gene flow patterns can be inferred from haplotype networks that connect each particular haplotype through mutational steps. This enables assignment of extant haplotypes to an ancestral population, while differentiating between ancestral polymorphisms and migration. This distinction is particularly useful when analysing species that have diversified or diverged quite recently, as is the case for the majority of cultivars (Londo *et al.* 2006). On the other hand, Popgraph draws from tools generated by landscape genetics that allow for the differentiation between isolation by distance and long distance migrations, which are phenomena that can underlie genetic differentiation among populations (Dyer & Nason 2004). These approaches explicitly incorporate geographical information to assess the contribution of physical space in structuring genetic diversity (Manel *et al.* 2003; Dyer 2009).

In the present study, we complement these types of historical gene flow analyses with estimates of ongoing gene flow using transgenes. While gene flow estimation is instrumental in the analysis of the genetic structure of populations, it should be complemented with a direct assessment of pollen and seed dispersal rates that impact on the natural patterns of gene flow. Otherwise, the consequences of dispersal-related life history variation among populations—and, hence, gene flow itself—will remain poorly quantified (e.g. Palstra *et al.* 2007). Therefore, we have also pursued the analysis of landscape features that can impact the genetic structure of populations by documenting the metapopulation structure of cotton in Mexico.

Metapopulations are assemblages of populations that exist in a balance between extinction and colonization (Levins 1969; Hanski & Gaggiotti 2004 and references therein). For plants, several criteria have been proposed that further constrain this metapopulation concept (Hanski 1998; Freckleton & Watkinson 2002), including: (i) that suitable metapopulation habitats are in spatially separated patches; (ii) that all patches can become extinct but they cannot do so at the same time; and (iii) that recolonization of each patch after local extinction is possible (Honnay *et al.* 2009).

The complex of wild and cultivated cotton populations in Mexico is an ideal system with which to address the role of metapopulation dynamics on recent and historical gene flow patterns, and on the genetic structure of populations. These studies are also instrumental for breeding and conservation programs for crops at their CCO. The germplasm of current cultivated cotton originated in Mesoamerica, where it was semi-domesticated in pre-Hispanic times (Tehuacán Valley, Mexico, dated around 5500–4300 BP; Smith & Stephens 1971). Previous studies used allozymes and RFLP data to identify possible venues of cotton domestication and to assess broad range genetic diversity (Wendel & Albert 1992; Brubaker & Wendel 1994). However, although cultivated cotton varieties are the most important source of natural fibre and the third source of oil in the world (FAOSTAT 2009), only two varieties (*G. hirsutum* var. *yucatanense*; called TX2094 and Deltapine 14; Delta and Pine Land Co; Applequist *et al.* 2001) have been used as reference for wild germplasm. Thus, broadening the genetic studies of wild populations of *G. hirsutum* will increase the success of breeding strategies focused on generating varieties adapted to extreme environments.

The *Gossypium* genus originated from African relatives between 12.5 (Seelanan *et al.* 1997) and 25 (Wendel & Albert 1992; Wendel *et al.* 2010) million years ago, and its salt-tolerant seeds enabled its spread around the world (Stephens 1966; Seelanan *et al.* 1997). Only four out of more than fifty *Gossypium* species have been domesticated (Wendel *et al.* 2009): two diploids in Asia and Africa (*G. herbaceum* and *G. arboreum*) and two tetraploids in America (*G. hirsutum* and *G. barbadense*). Current diploid and allopolyploid *Gossypium* species on the American continent cannot hybridize amongst themselves (Beasley 1940, 1942). Cotton is mainly self-pollinated, although cross-pollination may rarely occur (Stephens & Finkner 1953; Simpson 1954; McGregor 1976), and gene flow occurs via seed dispersal by water (Stephens 1966), and probably by wind and birds. In Mexico, GM cotton has been cultivated since 1996 and 172 000 ha were approved for sowing in 2009 (SAGARPA 2010). Despite the extent of GM cotton cultivation,

the dispersal of transgenes into non-GM and wild cotton has not yet been evaluated.

Given the complex history of the *Gossypium* genus and its capability for long distance migration, we first evaluated the geographical structuring of *G. hirsutum* populations in Mexico by generating a potential distribution based on climatic data. We hypothesized that geographic barriers have affected long distance gene flow among wild *G. hirsutum* populations, rendering a genetic structure that does not conform to an isolation-by-distance pattern across the area as a whole. We then documented historical gene flow using chloroplast microsatellite data to construct a haplotype network. Lastly, we used transgenes as markers to assess whether recent gene flow has taken place and if its patterns and dynamics conform to our historical inferences.

Materials and methods

Assessment of wild cotton populations and modelling of a potential distribution map

We selected populations of wild *Gossypium hirsutum* to be collected for this work by first performing an analysis of hundreds of historical specimens at the *MEXU* National Herbarium and *XAL* Herbarium. Twenty accessions were used that were clearly referenced as wild specimens and whose geographical reference fell within the formerly established natural habitats of this species. Concomitantly, we used the collections made by Paul A. Fryxell between 1968 and 1975 to guide our field search for wild populations. The specimens collected by Fryxell had clear features of wild cotton, as well as a precise description of both the habitat and location. Based on previous reports (Fryxell 1979; Wendel & Albert 1992; Applequist *et al.* 2001), we used the following objective criteria to classify a cotton plant as wild: (i) it is present in the expected habitat and distribution for the species' wild populations; (ii) it is a perennial shrub or tree, and (iii) its fruits have less than 22% lint content. We also delimited our unit of study, considering a population as comprised by a set of individuals that may potentially cross-pollinate among themselves and that are set at a distance of a maximum of 14 km among them. This distance criterion was set as a conservative limit, because this is the maximum pollinator (honeybee) movement range reported to date (Beekman & Ratnieks 2000).

We characterized the ecological niche for this species, based on 185 collection points of wild cotton plants surveyed between 2002 and 2007. We used the niche model proposed by Wiley (Wiley *et al.* 2003) and analysed our data using the GARP program (Genetic Algorithm for Rule-set Production; Scachetti-Pereira 2001), which incorporated 23 bioclimatic covers from Worldclim, with a convergence limit of 0.01%, a 5% of omission, and a 10% commission threshold. Models were selected using the methodology proposed by Anderson *et al.* (2003).

The potential distribution map of *G. hirsutum* wild populations in Mexico was delimited through comparison with cartography from the Biogeographic Regions of CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad 1997). The predicted areas of distribution of wild cotton were validated through field inspections of places diagnosed to contain *G. hirsutum* wild populations according to the potential distribution maps, but where no former collections had been undertaken or where no entries were available at any database consulted. With these data, we analysed the population structure using a metapopulation scheme (see 'Results' section).

Cotton seed collection

Cotton seeds were collected between 2002 and 2008 in the identified wild populations of this species. The size of surveyed populations varied in number from 4 to 24 plants, with 1 up to <14 km separating individuals within a population. In total, 336 individual plants distributed in 36 populations were collected (Fig. 1).

Additionally, seeds from commercial cotton cultivars from Sonora, Mexicali, Chihuahua (Mexico), Texas, Virginia (USA), Argentina, Brazil, India and Egypt, were used to assess genetic diversity. We also collected seed from populations present outside the potential distribution area: Cuautla (18.89 N, −99.97 W), Tepoztlán (18.97 N, −99.09 W), Cuernavaca (18.87 N, −99.205 W; in the state of Morelos), Durango (23.18 N, −104.52 W) and Sonora (28.80 N, −110.57 W). All of these were considered feral populations because they have more than 25% of lint and are far away from potential distribution areas of wild cotton.

Laboratory procedures

DNA and chloroplast microsatellite analyses. Collected cotton seeds were germinated in growth chambers with a 12 h/30 °C light and 12 h/20 °C darkness regime, in 80–90% humidity. Genomic DNA was isolated from young seedling leaves using a modified CTAB method from Sul & Korban (1996; see Table S1).

DNA sequences were amplified through PCR using two specific primer sets for *G. hirsutum* chloroplast microsatellites (AF351292 $(GAA)_9$ and AF351313 $(CA)_{12}$, from Reddy *et al.* 2001). Additionally, ten PCR primer sets were used for the analysis of simple sequence

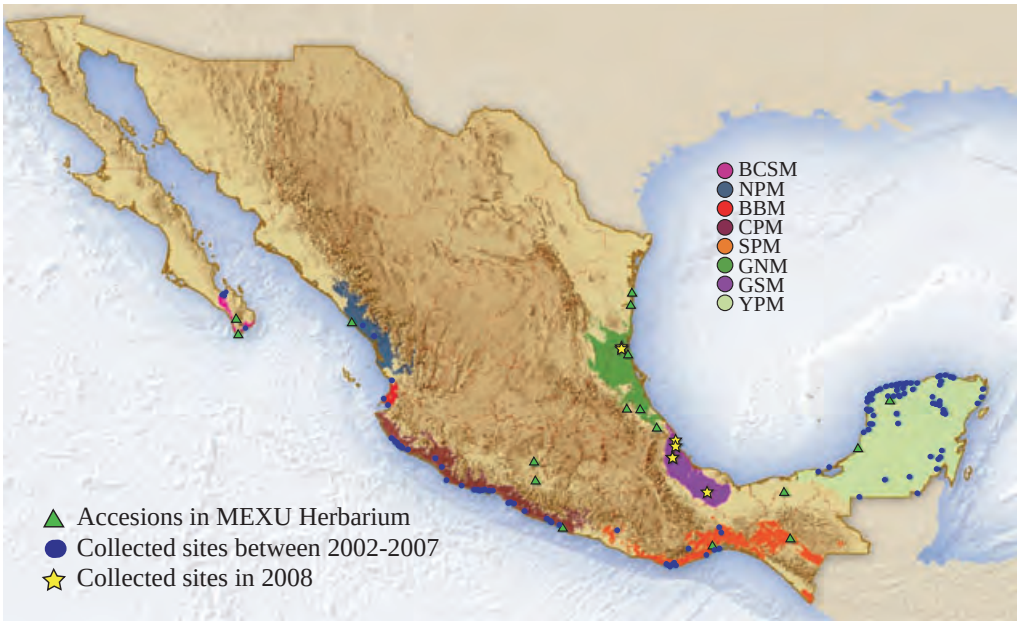

Fig. 1 Map showing collection sites, potential distribution area and metapopulations of *G. hirsutum* in Mexico. Symbols: green triangles: cotton collections identified by Fryxell at the MEXU herbarium; blue circles: 2002–2007 cotton collections; yellow stars: 2008 collections discovered with the use of the potential distribution map. Metapopulations are coded as follows: Baja California Sur (BCSM): fuchsia; North Pacific (NPM): grey; Banderas Bay (BBM): red; Central Pacific (CPM): burgundy; South Pacific (SPM): orange; Gulf North (GNM): dark green; Gulf South (GSM): purple; and Yucatán Peninsula (YPM): lime.

repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms (CCMP1–CCMP10 from Weising & Gardner 1999). The PCR procedure and individual conditions are shown in Table S1. DNA fragments were sequenced on an ABI Prism 3730xl Analyzer at the High-Throughput Sequencing and Genotyping Unit in the University of Illinois.

Immunoassays to detect the presence of transgenes in wild cotton populations. A total of 270 individual cotton seeds from 36 populations ($N \geq 20$ seeds per population; see Table 1) were individually analysed for transgene presence via immunoassays for the most common recombinant proteins present in cultivated cotton for which ELISA kits were available (Cry1Ab/Ac, Cry2A, CP4-EP-

Table 1 Presence of recombinant proteins in *G. hirsutum* metapopulations

Metapopulation	Populations	*N*	Positive seeds	Positive 1 protein	Positive 1 + proteins
BCS (S of BCS)	*2*	17	0	0	0
North Pacific (Center and S of Sinaloa and N of Nayarit)	*3*	37	25	19	6
Banderas Bay (SW Nayarit and NW of Jalisco)	*2*	15	0	0	0
Center Pacific (Coastal line of C and S of Jalisco, Colima, Michoacán and NW and C of Guerrero)	*6*	24	0	0	0
South Pacific (SE of Guerrero, Coastal line of Oaxaca, CW, C and South tip of Chiapas)	*8*	44	13	13	0
Yucatán Peninsula (Quintana Roo, Yucatán, Campeche and NE and E of Tabasco)	*11*	88	0	0	0
Gulf South (C and SE of Veracruz)	*3*	21	14	12	2
Gulf North (N of Veracruz, E of San Luis Potosí and S of Tamaulipas)	*1*	24	14	0	14
Total	36	270	66	44	22

The region comprised within each metapopulation is described in parentheses; the number of wild cotton populations in each metapopulation is presented in column two. Symbols: *N*: total number of seeds analysed per metapopulation; positive: total number of seeds positive for recombinant protein presence; positive 1 protein: number of seeds positive for only one recombinant protein; positive 1 + protein: number of seeds positive for more than 1 and up to 4 different recombinant proteins (see text for a complete description).

SPS and PAT/Bar). The embryo of each seed was separated from its seed coat and divided into four pieces with a surgical knife. Each piece was placed in a 2-mL microcentrifuge tube for separate homogenization with an appropriate volume of extraction buffer. Each sample was analysed using duplicate assays in each ELISA plate. Immunoassays were conducted according to the manufacturer's instructions. The ELISA plates were read in a spectrophotometer at 450 nm for proteins PAT/bar, Cry2A and CP4-EPSPS-event NK603 (Envirologix™ plates) and at 650 nm for proteins CP4-EPSPS and Cry1Ab/Ac (Agdia® plates).

We considered a sample to be positive only when its absorbance was equal to or above a reading three standard deviations above the average intensity of all negative controls and blank samples. At least one duplicate of a blank (extraction buffer), one negative control, and one positive control were included in each ELISA plate.

Data analyses

Molecular diversity. We determined the number and frequency of all unique chloroplast DNA haplotypes and estimated molecular diversity using Arlequin v3.5 (Excoffier & Lischer 2010). We used the rarefaction approach (using ADZE; Szpiech *et al.* 2008) to see if heterogeneous population sizes could affect the estimation of genetic diversity among populations and also to generate estimates that would be comparable among different populations (Petit *et al.* 1998; Kalinowsky 2004).

Genetic structure and gene flow analyses. We examined population structure by performing a Bayesian spatial analysis using the program BAPS 5.1 (Corander *et al.* 2008), which uses stochastic optimization to find the optimal partition. Simulations were run from $K = 2$ to $K = 10$ with 100 replicates for each K.

We sought evidence for isolation by distance and/or long-distance dispersal events using Population Graph (GeneticStudio software; Dyer 2009). This is a graph-theoretic approach that analyses how genetic variation is distributed across the investigated landscape, by plotting migration and enabling the assessment of the dependence or independence of evolutionary trajectories among populations. Within a graph, populations are represented as nodes and the genetic covariation among populations determines the topology. The pattern of connections between populations is estimated conditional on the entire data set. The pattern can be used to test for isolation-by-graph-distance, where in an extreme case, if covariance between two populations equals zero, no connection is drawn (IBGD; Dyer & Nason 2004). Plotting the Population Graph onto a map also allows the inferred population pairs to have 'extended edges', 'normal edges', or 'compressed edges', which imply that genetic distance is either higher, equal to, or lower, respectively, than the one expected by geographic data (Dyer 2009).

We investigated the evolutionary history and relationships among the haplotypes found in this study and differentiation of the ancestral polymorphism and gene flow by constructing a minimum-spanning network of haplotypes using TCS 1.21 (Clement *et al.* 2000). We used the methods described by Templeton & Sing (1993) to break loops (ambiguous connections) within our network, while using predictions derived from coalescence theory (reviewed in Rosenberg & Nordborg 2002).

Distances between GM cotton release sites and wild cotton populations. Mexico was divided into over 80 000 hexagons, 25 km^2 each, to compare areas against experimental release centres. Centroids of these hexagons were used to calculate the distance between the release sites and the potential distribution model, with an error of 25 km. The sites where permits to release genetically modified cotton in Mexico have been granted (from 1996 to 2008) were plotted on a map of Mexico, under the assumption that all plots approved were actually planted (Fig. 4a). The minimum distance separating a granted GM cotton release site from all populations of wild cotton was determined (Table 2).

Results

Wild populations of G. hirsutum *in Mexico: potential distribution and actual metapopulation structure*

A potential distribution map was generated using computational and geographic tools (GARP). This map was based on a comprehensive survey of existing wild *G. hirsutum* populations comprising 185 collection points (recorded between 2002 and 2007) distributed in 36 populations (blue points in Fig. 1). The potential distribution is plotted in Fig. 1 and represents those areas that had over 75% of confidence of translating into the actual wild cotton distribution, according to our survey data. Thus, the actual distribution area for this species may possibly be even broader than that considered here. Nevertheless, the fact that all predicted populations were either corroborated or led to the finding of new populations helped us to validate the precision of the ecological niche prediction model used here. The potential distribution map identified seven new populations along the Gulf of Mexico in 2008 (yellow stars in Fig. 1). In previous years, without the guidance of this model, efforts to find wild populations in this area had proved unsuccessful.

During the 7-year fieldwork period (2002–2008), we observed that 85% of wild cotton populations were in coastal ecosystems and some were in low dry forests. Cotton plants were in populations of 4 to 20 individuals (5 was the mode).

The spatial distribution and the ecological setting of the populations investigated here suggest the existence of eight discrete bioclimatic areas. These are separated by intermediate zones that lack adequate climatic and ecological conditions for *G. hirsutum* to grow, and that effectively form geographical barriers to seed and pollen flow. Each discrete area described here is considered to be a metapopulation because cotton plant populations were discontinuous due to the discrete occurrence of favourable habitats. Furthermore, each metapopulation was separated from one another by at least 150 km or was isolated by evident geographical barriers.

The eight metapopulations proposed here are: Baja California Sur (BCSM), North Pacific (NPM), Banderas Bay (BBM), Center Pacific (CPM), South Pacific (SPM), Yucatán Peninsula (YPM), Gulf South (GSM) and Gulf North (GNM; Fig. 1). Although we lack quantitative dynamic data for all of the populations surveyed, the number of plants per population, as well as the number of populations that form a metapopulation, varied substantially (Table 1). In the northern part of the country, the maximum number of populations per metapopulation is three (BCSM, NPM, BBM and GSM). In the south of Mexico, three metapopulations have six, eight, and eleven populations (CPM, SPM and YPM, respectively). With regard to suitable habitats for cotton growth within metapopulations, the YPM has the largest contiguous range of suitable habitats and bears the largest populations. It is also the most genetically diverse.

Genetic variation, historical gene flow, and population structure of G. hirsutum *in Mexico*

Overall, genetic variation among wild metapopulations of *G. hirsutum* is high (He = 0.894 ± 0.01). We found a total of 46 haplotypes, 78% of which are unique to a particular metapopulation (Fig. 2). The highest haplotype diversity was found in BBM (0.94) and YPM (0.93; for haplotype diversity between metapopulations, see Table S2). The remaining metapopulation diversity ranges between values of 0.6 and 0.8, except for GSM, which is exceptionally low (0.34). In contrast, the analysed commercial cotton seeds and inferred feral populations have only one haplotype (number 2; Fig. 2a). The only exception to this trend is the feral population in Cuernavaca, Morelos, where two haplotypes were found (2 and 25; Fig. 2a).

The 46 haplotypes found in this study group into six distinct lineages, of which those of BBM and YPM are well differentiated (Fig. 2a). This haplotype network has a complex topology, where some populations with unique lineages have haplotypes that are not sampled,

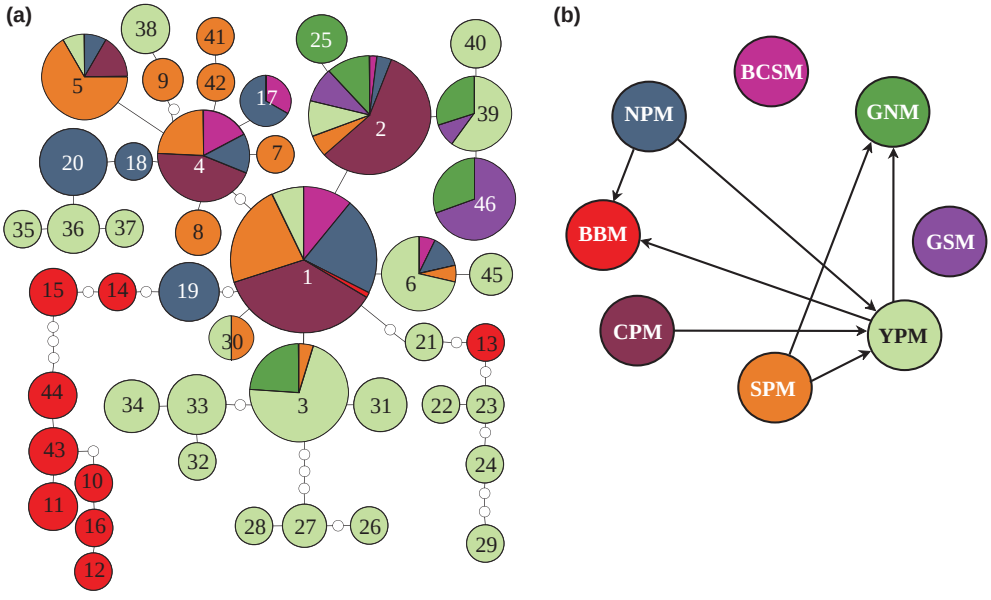

Fig. 2 Haplotype network and historical gene flow in wild *G. hirsutum* metapopulations. (a) Haplotype network. Haplotypes documented in this work are depicted in circles; sizes of nodes show the frequency of a particular haplotype while colours represent the presence of a particular haplotype within each metapopulation. (b) Historical gene flow patterns among metapopulations, as inferred from the haplotype network (metapopulation colour-codes and labels are as in Fig. 1).

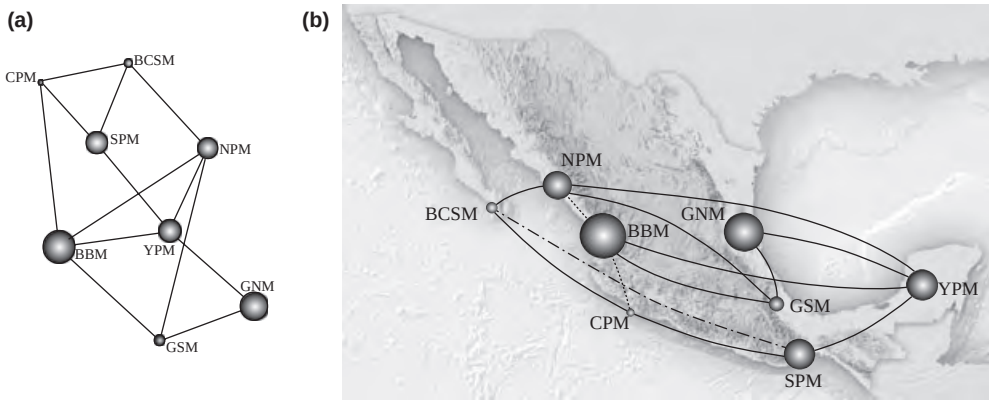

Fig. 3 *Popgraph* analysis showing significant connections among wild cotton metapopulations. (a) Three-dimensional *Popgraph* representing the genetic covariance among metapopulations of *G. hirsutum,* based on chloroplast markers. The length of a line between any two metapopulations is proportional to their covariance; within-metapopulation genetic variance is proportional to sphere size. (b) Geographic distances among metapopulations and their relation with the *Popgraph* analysis shown in (a). Edges that are significantly longer (– - – - –), shorter (—) or congruent (——) with the predicted genetic covariance with respect to geographic distance are plotted. Names are as in Fig. 1.

while shared haplotypes among several populations appear to be ancestral. Derived haplotypes are generally unique. The unique haplotype of cultivated cotton (2) is shared and frequent in almost all metapopulations. Haplotypes 35, 36, and 37 show ancient gene flow, while 38 and 5 seem to have recently migrated from the YPM; lastly, haplotype 13 shows evidence of ancestral gene flow (Fig. 2b).

We estimated the genetic structure among the surveyed wild cotton populations by modelling our data using the BAPS approach. We found that the optimal number of clustered groups was eight; thus, the description of each cluster by this algorithm is consistent with the metapopulation scheme derived from the potential distribution analysis used above. Furthermore, the rarefaction approach was consistent with the Population Graph tool, as the diameter representing the genetic variation is not correlated with the sample size per population (Fig. 3).

Given the inferences of genetic variation and metapopulation genetic diversity presented above, which are the result of historical events, we addressed the question of whether contemporary long distance gene flow has taken place, by evaluating transgene flow.

Recent long distance gene flow: presence of transgenes in wild cotton metapopulations

The potential for long and short-distance ongoing transgene flow that could be occurring from GM cotton plants to native wild cotton populations was evaluated through plotting the frequency distribution of the distance between the GM cotton parcels and the nearest wild cotton population (Table 2). In this analysis, we found that 1.4% of 5985 permits to sow GM cotton issued by the pertinent Mexican authority between 1996 and the beginning of 2008, fall within the area of distribution of two metapopulations of wild cotton (NPM and GNM), while 4.2% are within a 300-km radius from three metapopulations (NPM, GNM and GSM). The remaining 94.4% of GM field releases approved are over 300 km apart from all wild cotton metapopulations (Table 2).

We identified actual transgene flow by assessing the presence of recombinant proteins in wild cotton populations through ELISA tests. The immunoassays yielded 66 positive seeds out of 270 seeds tested (24.4%) for at least one recombinant protein (Table 1). These positive cases were distributed among four metapopulations (Fig. 4): NPM (25/37; 67.6%), GNM (14/24; 58.3%), GSM (14/21; 66.7%) and surprisingly, SPM (13/44; 29.5%). The latter is at a lineal distance of 755 km from the southernmost and nearest approved GM cotton plot. Furthermore, 3 out of 3 populations comprising the NPM had positive testing plants for transgene presence; 1 out of 1 in GNM; 2 out of 3 in GSM and 3 out of 7 in the SPM. Interestingly, two-thirds of all positive samples yielded positive results for a single recombinant protein, while one-third did so for two and up to four different transgene-codified proteins (Table 1).

Of the 66 positive seeds, 15.9% had the haplotype common to the domesticated cultivars (haplotype 2). In the GNM, 6 out of 14 positive seeds for Cry1Ab/Ac have haplotype 2. In the GSM, three out of five individuals positive for CP4-EPSPS had this haplotype. In the NPM, two seeds positive for Cry1Ab/1Ac and Cry2A shared this haplotype. In the SPM, none of the positive seeds for recombinant proteins had this haplotype.

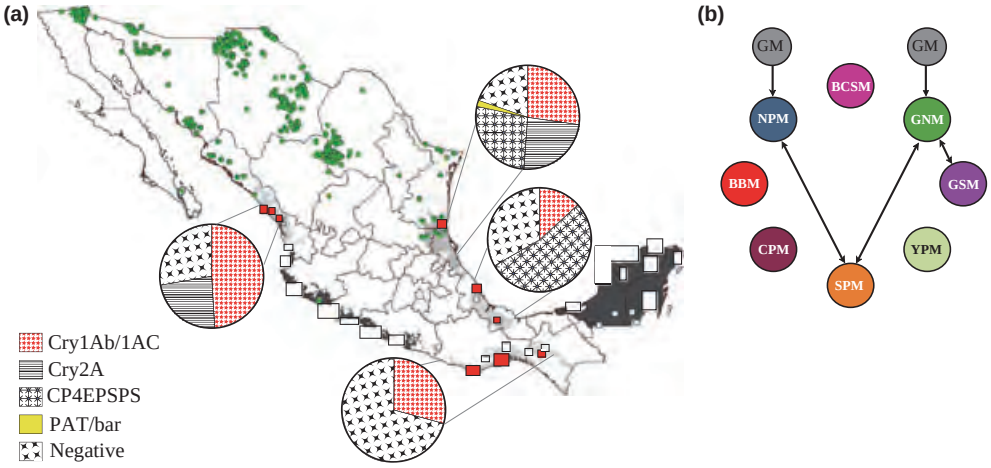

Fig. 4 Contemporary gene flow among cotton metapopulations as inferred by transgene presence. (a) Map of Mexico showing the regions where GM cotton cultivars have been approved for planting, as well as wild cotton metapopulations and populations positive for recombinant protein presence. GM cotton cultivation sites are plotted as green circles; metapopulations without recombinant proteins (BCSM, BBM, CPM, and YPM) are coloured in dark grey; metapopulations with recombinant proteins (NPM, SPM, GNM and GSM) are in pale grey; wild cotton populations with transgene presence are plotted as red squares while populations without transgenes are depicted as white squares. Pie charts with the frequency of particular recombinant proteins are set aside each transgene-harbouring metapopulation. (b) Diagram showing possible venues of present gene flow between GM cultivars and some wild cotton metapopulations. Arrows show the probable trajectories of transgene flow.

Discussion

In this study, we have shown that long distance gene flow has taken place among *G. hirsutum* wild populations, both historically and recently. Evidence from the population genetic analyses and the metapopulation scheme suggests that geographical barriers can hinder population structuring, but not sufficiently to suppress migration among metapopulations.

Cotton metapopulations: current distribution, metapopulation dynamics, and changes in land use

In this work, we propose the existence of eight distinct wild cotton metapopulations in Mexico. While the standards utilized in this work to define metapopulations are qualitative, they are consistent with the delimitation criteria put forward by other scholars (Hanski 1998; Freckleton & Watkinson 2003; Honnay *et al.* 2009). In line with metapopulation theory, we found that 34% of the historically characterized *G. hirsutum* populations continue to dwell in their original geographic zones. Local extinction and recolonization was also observed in 68 of 171 collection points surveyed for which at least two visits were performed during this work.

This dynamic turnover could be favoured by the fact that 55% of surveyed wild populations live in disturbed areas. This suggests, based on Fryxell's and others' previous assessments of wild cotton populations, that a process of habitat alteration due to human and abiotic perturbations (changes in land use, as well as hurricanes and tropical storms) has taken place. These phenomena have shaped the species' habitat along the Pacific and Gulf of Mexico coastal lines. The survival of these populations in disturbed areas is probably related to the ability of this species to grow well in places with low plant cover and high solar exposure, as well as having a perennial habit, being sexually mature during the first year of life, having populations composed of plants at different life stages, and presenting long distance seed dispersal. Nevertheless, while habitat perturbations have not affected all cotton populations, they could drive a significant number of them to extinction, especially in a scenario where extreme changes in land use would hinder recolonization. This could be the case for the coastal region of the Gulf of Mexico (GNM and GSM), which has been subjected to land use changes due to promotion of agriculture and cattle grazing areas (GNM) or to the establishment of hotel resorts that deplete coastal dunes (GSM). This probably accounts for the smaller number of wild cotton populations documented in this study for that part of the country.

While the dynamics currently affecting metapopulation structuring are probably significantly influenced by human activity, the structure unveiled in this work can only be explained in evolutionary time. The modelling of the ecological niche for *G. hirsutum* populations in Mexico was also based on data obtained from actual wild cotton populations. This confers a more precise

Table 2 Linear distances between GM cotton release-sites and wild cotton metapopulations

Cotton growing region	Minimum distance between GM cotton plot and a wild cotton metapopulation (km)	Number of granted permits (1996–2008)
Tamaulipas and Sinaloa	0	85
Tamaulipas, Sinaloa and South Sonora	1–100	152
	101–200	42
	201–300	56
Comarca Lagunera	301–400	919
	401–500	1200
South Chihuahua	501–600	378
	601–700	274
North Chihuahua	701–800	1375
North Sonora	801–900	210
	901–1000	210
Mexicali, Baja California Norte	1001–1100	1084

input for distribution inference algorithms such as GARP, than was obtained by previous studies where this distribution was inferred using data from cultivated cotton (Rogers *et al.* 2007).

Ancestral gene flow among cotton metapopulations and cotton cultivars

We evaluated historical gene flow using chloroplast microsatellites (maternally inherited alleles) to detect historical gene flow through seed migration. Our data indeed suggest long distance seed migration that is consistent with previous suggestions of the potential for seed dispersal through marine currents, given the viability of seeds subjected to prolonged incubation periods in salt water (Stephens 1958). This finding is consistent with the signature of molecular markers during chromosomal speciation (Wendel 1989; Wendel & Albert 1992; Andersson & de Vicente 2010). Interestingly, when assessing recent long distance gene flow through transgene presence in wild *G. hirsutum* populations, we find high migration rates ($m = 66/270 = 0.24$), but this does not seem to be due to seed migration, since only 15.9% of the plants that were positive for transgene presence have the haplotype common to domesticated cotton used to generate transgenic lines (haplotype 2). This observation could imply low seed migration out of GM fields. Nevertheless, once a single or a few transgenic individuals are dispersed into particular wild populations, they produce pollen that may fertilize local wild plants. Since transgenes are inserted within the nuclear genome, they can be dispersed both via pollen or seed.

As cotton was domesticated centuries ago, ancient gene flow between domesticated cultivars and its wild relatives could probably have occurred historically via seed dispersal, favoured by human activities and environmental phenomena. Thus, some of the genetic patterns observed could be the product of these types of ancestral events. Nevertheless, we assumed that the observed genetic structure is affected by historic gene flow events among cultivated and wild cotton and we repeated the haplotype analysis, this time eliminating haplotype 2 (the only haplotype in cultivated specimens). We did not find significant changes with respect to the structure reported here (data not shown).

The haplotype network that we have put forward has helped us to distinguish ancient polymorphisms from recent gene flow events. Furthermore, these approximations have been complemented by the estimation of recent gene flow using transgenes as markers in extant wild cotton populations (Fig. 4).

Transgenes in wild cotton metapopulations

Fifteen years after the introduction of GM cotton cultivars into Mexico, we have documented the presence of recombinant proteins in wild cotton populations at its CCO (see Fig. 4a). We assayed recombinant protein activity using ELISA kits available in Mexico. These enabled us to detect 18 out of 21 approved events (CERA 2010) among individuals of wild cotton populations. The remaining undetectable events (3) have been scarcely sown. The traits that have been introduced, alone or in different combinations, into currently sown cotton varieties through genetic engineering include Lepidoptera resistance (*Cry1Ab/Ac, Cry2Ac, Cry1F* and *vip3A*), herbicide tolerance (CP4-EPSPS), and antibiotic resistance (*PAT/Bar*, *nptII* and *aph4*; Traxler & Godoy-Avila 2004).

The combinations of recombinant proteins detected in this study differ among metapopulations, which suggests that each combination could have been the result of independent and multiple transgene flow events into the Mexican wild cotton populations. This observation is additionally supported by the fact that 84.1% of seeds that tested positive for transgene expression had a haplotype other than the one present in the cultivars (2). Since cotton is assumed to be self pollinated, transgene flow must also have occurred mostly via seed and secondary cross-pollination events (Dyer *et al.* 2009).

The combinations of transgenes found within metapopulations and the possible transgenic events from which they could have originated are as follows: in PNM, plants expressing Cry1Ab/Ac, could have originated from event MON531; in GSM, CP4-EPSPS protein could involve either MON88913 or MON1445/1698; for

the GNM and GSM metapopulations, the recombinant protein combinations found -Cry1Ab/Ac and CP4-EPSPS- suggest that the most likely transgenic event could be MON531 × MON1445. For GNM, the Cry1Ab/Ac, Cry2Ac, and CP4-EPSPS proteins could originate from MON15985 × MON1445 or MON88913. These events were approved for planting in Mexico between 1996 and 2003. In the case of seeds positive for transgenes that harbour haplotype 2 and have transgene combinations consistent with a commercial transgenic variety (15.9%), we could be detecting feral GM cotton plants that have dispersed into suitable habitats, but, given the environmental conditions, do not grow to resemble their cultivated counterparts.

In contrast, we found some transgene combinations that cannot be explained as primary gene flow events, given the transgene combinations present in the currently available GM cotton lines. This is the case of a seed from GNM that expresses all four recombinant proteins assayed. This finding suggests that recurrent gene flow events and gene stacking could already have occurred in this metapopulation. In contrast, some seeds from NPM and SPM only expressed the Cry2Ac protein, which is not contained individually in any commercial event. This phenomenon could involve independent segregation of transgenes from some lines and a later introgression into wild cotton. Alternatively, it could represent transcriptional or post-transcriptional gene silencing of either the CP4-EPSPS or Cry1Ab gene/transcripts that are present in all commercially available lines expressing Cry2Ac. In order to distinguish between these hypotheses, DNA-based analyses should be undertaken using transgene specific primers, both from the DNA of the seed and that of the mother plant.

Detected transgenes were aggregated in space ($p = 0.001$). This type of distribution could be favoured by the dynamics of plant metapopulations. In the particular case of wild cotton, populations where recolonization has taken place have few plants, and thus can be subject to a genetic bottleneck and to genetic drift. Transgene frequencies and spatial patterns documented here also suggest that transgene introduction is relatively recent and has not been fixed in all metapopulations. These findings could imply that these new alleles do not confer a high selective advantage. In the particular metapopulations where not all populations are positive for transgene presence (GSM and PSM), but are in close proximity to some of the positive populations, three hypotheses would explain the intermixing of positive and negative populations. Firstly, negative populations are more recent than positive ones and are the product of colonization/recolonization from wild (non-transgenic) seed. Alternatively, these populations did have transgenes, but the transgenes were eliminated by genetic drift or selection. Finally, transgenes in these populations may exist but are silenced or have not reached some populations simply due to random events.

Our transgene data confirm that long-distance gene flow is preeminent in wild cotton at its CCO. Given present day management practices, some means of seed movement at long distances include the accidental dispersal of cotton seed intended for animal feed. We observed this happening in trucks from the USA to the centre-south of Mexico. This phenomenon takes place because seeds that are separated from their fibre are later sold as animal feed without being previously mashed into a 'cake'. This venue for GM seed dispersal could very well be occurring for GM seed processed in Mexico, because very little attention is paid to the disposal of this seed once the fibre has been removed. Given the documented patterns, future studies should address the possible scenarios to be expected in terms of transgene flow and accumulation, as well as the consequences these may have for wild cotton conservation at its CCO, as has been documented for maize in Mexico (Dyer & Taylor 2008; Piñeyro-Nelson *et al.* 2009).

In this study, we found no correlation between transgene presence and loss of genetic diversity. Nevertheless, in order to explore whether the presence of transgenes could have consequences in wild cotton populations, sustained and long-term analyses should be pursued. In particular, biomonitoring studies that assess the consequences of both transgene and foreign germplasm introduction into wild metapopulations of *G. hirsutum*, should be undertaken (see, for example, Meirmans *et al.* 2009).

This study confirms that ELISA-based analyses are useful when assessing the presence of transgenes in wild cotton metapopulations. Nevertheless, future studies should also consider DNA-based detection methods to corroborate our findings, as well as to determine the specific events involved. This multiple-technique approach has been suggested in other studies dealing with transgene detection at CCO (Serratos-Hernández *et al.* 2007; Piñeyro-Nelson *et al.* 2009).

Lastly, gene flow from cultivated cotton can put the wild germplasm of several *Gossypium* species at risk. Evidence from previous investigations suggests that *G. tomentosum* (in Hawaii), *G. mustelium* (in Brazil) and *G. darwinii* (in Galapagos) are at risk of extinction as a result of hybridization with domesticated tetraploid cotton (Ellstrand 2003; Andersson & de Vicente 2010). In some cases, interspecific hybrids (*G. hirsutum* × *G. barbadense*) may act as genetic bridges for gene transfer from domesticated cotton to other wild relatives (*G. darwinii*; Ellstrand 2003; Andersson & de Vicente 2010). As a con-

sequence, conservation programs should include all *Gossypium* tetraploid species.

Conclusions

The interplay of historical long distance gene flow and geographic barriers in Mexico has shaped the genetic structure of extant populations of *G. hirsutum*. Extinction and recolonization events in particular populations have hindered genetic homogenization among metapopulations.

Potential distribution analyses and molecular markers independently show the existence of eight metapopulations. We were able to record intense dynamics of recent local extinctions and colonizations that go back to the collections made by Paul Fryxell. In spite of their integrity, these metapopulations are connected through long distance migration events. In particular, through the assessment of transgene presence, we were able to detect recent gene flow, which supports the connectivity of these metapopulations. This scenario of long distance colonization, the existence of metapopulations, and the presence of transgenes at its CCO calls for conservation efforts both *in situ* and *ex situ*. These types of endeavours rely upon preservation of the habitat currently occupied by wild cotton plants or on opening up of new habitats for wild cotton colonization. The metapopulation perspective must be kept in mind (Meirmans *et al*. 2003), as 'metapopulation persistence relies on the existence of a certain amount of suitable but currently unoccupied habitat' (Freckleton & Watkinson 2002, 2003). Coastal dunes appear to be particularly important areas in this respect. In addition, demographic studies of wild cotton populations, documentation of spatio-temporal patterns of seed and pollen dispersal, and rates of cross-pollination among wild individuals should also be the basis for guiding these conservation efforts.

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Data accessibility

Microsatellite data available in DRYAD: doi:10.5061/dryad.rd8fn

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Description of DNA extraction procedure and PCR conditions

Table S2 Pairwise comparison matrix of genetic distance and genetic diversity among metapopulations

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Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years

Charles M Benbrook

Abstract

Background: Genetically engineered, herbicide-resistant and insect-resistant crops have been remarkable commercial successes in the United States. Few independent studies have calculated their impacts on pesticide use per hectare or overall pesticide use, or taken into account the impact of rapidly spreading glyphosate-resistant weeds. A model was developed to quantify by crop and year the impacts of six major transgenic pest-management traits on pesticide use in the U.S. over the 16-year period, 1996–2011: herbicide-resistant corn, soybeans, and cotton; *Bacillus thuringiensis (Bt)* corn targeting the European corn borer; *Bt* corn for corn rootworms; and *Bt* cotton for Lepidopteron insects.

Results: Herbicide-resistant crop technology has led to a 239 million kilogram (527 million pound) increase in herbicide use in the United States between 1996 and 2011, while *Bt* crops have reduced insecticide applications by 56 million kilograms (123 million pounds). Overall, pesticide use increased by an estimated 183 million kgs (404 million pounds), or about 7%.

Conclusions: Contrary to often-repeated claims that today's genetically-engineered crops have, and are reducing pesticide use, the spread of glyphosate-resistant weeds in herbicide-resistant weed management systems has brought about substantial increases in the number and volume of herbicides applied. If new genetically engineered forms of corn and soybeans tolerant of 2,4-D are approved, the volume of 2,4-D sprayed could drive herbicide usage upward by another approximate 50%. The magnitude of increases in herbicide use on herbicide-resistant hectares has dwarfed the reduction in insecticide use on *Bt* crops over the past 16 years, and will continue to do so for the foreseeable future.

Keywords: Herbicide-resistant crops, Herbicide-tolerant soybeans, Glyphosate, 2,4-D, *Bt* crops, Genetically engineered corn, Roundup Ready crops, Biotechnology and pesticide use, Glyphosate resistant weeds

Background

Public debate over genetically engineered (GE) crops is intensifying in the United States (U.S.), driven by new science on the possible adverse health impacts associated with herbicide-resistant (HR) crop pesticide use, and the rapid spread of glyphosate-resistant weeds. Still, many experts and organizations assert that GE crops have reduced, and continue to reduce herbicide, insecticide, and overall pesticide use. Fortunately, high quality and publically accessible U.S. Department of Agriculture (USDA) pesticide use data are available and can be used to track changes in pesticide use on crops containing GE traits. Moreover, the impacts of these traits on U.S. pesticide use trends are substantial and obvious, especially in recent years as a result of the growing number and geographical spread of glyphosate-resistant (GR) weeds.

Stable reductions in insecticide use in *Bt*-transgenic corn are also now in jeopardy as a result of the emergence of corn rootworm (CRW) populations resistant to the Cry 3Bb1 toxins expressed in several corn hybrids [1,2]. To combat this ominous development, some seed and pesticide companies are recommending a return to use of corn soil insecticides as a resistance management tool. There is a degree of irony in such recommendations, given that the purpose of Cry 3Bb1 corn was to eliminate the need for corn soil insecticides.

The emergence of herbicide-resistant genetically engineered crops in 1996 made it possible for farmers to use a broad-spectrum herbicide, glyphosate, in ways that were

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previously impossible. From 1996 through 2011, 0.55 billion hectares of HR corn (*Zea mays*), soybeans (*Glycine max*), and cotton (*Gossypium hirsutum*) were grown in the U.S. [Additional file 1: Table S7]. In 2011, an estimated 94% of the soybean area planted, 72% of corn, and 96% of cotton were planted to HR varieties, respectively, while about 65% of corn and 75% of cotton hectares in the U.S. were planted to *Bt* varieties [Additional file 1: Table S6].

Glyphosate-resistant, Roundup Ready (RR) crops now comprise the overwhelming majority of HR crops. RR crops were rapidly adopted because they provided farmers a simple, flexible, and forgiving weed management system, especially compared to systems reliant on the low-dose, persistent herbicide chemistries on the market in the late 1990s, such as imazethapyr (43% soybean hectares treated in 1996) and chlorimuron-ethyl (14% treated). From 1996 through 2008, HR crops resistant to herbicides other than glyphosate either disappeared from the market (e.g. bromoxynil HR cotton), or have been planted on relatively few hectares (e.g. glufosinate HR, LibertyLink cotton and corn).

Net reductions in pesticide use, encompassing changes in both herbicide and insecticide kilograms/pounds applied, are among the purported claims of GE crops [3-5]. Analysts assessing the impacts of *Bt* crops on insecticide use report reductions, or displacement, in the range of 25% to 50% per hectare [6]. A more recent study reports a 24% reduction [5]. On GE and non-GE corn since 1996, the volume of insecticides applied has declined, because of the pesticide industry-wide trend toward more biologically active insecticides applied at incrementally lower application rates.

The corn rootworm (CRW) has been the target of the majority of corn insecticide applications the last several decades. The average corn insecticide application rate in 1996 was about 0.76 kilograms of active ingredient per hectare (kgs/ha) (0.7 pounds/acre) and is less than 0.2 kgs/ha today (0.18 pounds a.i./acre) [Additional file 1: Table S12]. The two contemporary corn soil insecticide market leaders – tebupirimiphos and tefluthrin – are applied at average rates around 0.13 kgs/ha (0.12 pounds/acre). In 1996, the market leaders were chlorpyrifos and terbufos, insecticides applied at rates above 1.12 kgs/ha (1.0 pounds/acre) [Additional file 1: Table S12]. Obviously, planting *Bt* corn in 2011 reduced insecticide use less significantly compared to land planted to *Bt* corn in the late 1990s.

Few comprehensive estimates have been made of the impacts of HR crops on herbicide use. The USDA has not issued a new estimate in well over a decade; the USDA's Economic Research Service (ERS) reported an 3.7 million kg (8.2 million pound) decrease in pesticide use in 1998 as a result of GE corn, soybeans, and cotton [7], an estimate that is comparable to the present study's estimate of a 4.4 million kg (9.6 million pound) reduction [Additional file 1: Table S15]. A series of unpublished simulation studies have been carried out by the National Center for Food and Agriculture Policy (NCFAP). In a report covering crop year 2005, NCFAP projected that HR corn, soybean, and cotton reduced total herbicide use by 25.6 million kgs, compared to hectares planted to non-HR varieties [6]. Sankula's herbicide use estimates are based on observations of mostly university experts regarding "typical" herbicide use rates on farms planting HR versus non-HR varieties. The rates incorporated in Sankula's estimates often differ from those published for the same year by USDA's National Agricultural Statistics Service (NASS) [8]. NASS reported that an average 1.5 applications of glyphosate were made on HR soybeans in 2005, while Sankula assumes only 1.18 applications. Sankula's estimate of total herbicide use on RR soybeans in 2005, 1.15 kgs/ha (1.03 pounds/acre), is less than the NASS figure for glyphosate alone, 1.23 kgs/ha (1.1 pounds/acre). If true, Sankula's data suggests that essentially no other herbicides were applied to RR soybeans in 2005, when in fact the average soybean hectare in 2002 was treated with 1.66 herbicides according to NASS data.

This paper quantifies the impacts of GE crops on the kilograms of pesticides applied per hectare and across all GE hectares, drawing upon publicly accessible USDA data. The pesticide use impacts of the six major, commercial GE pest-management traits are modeled and then aggregated over the 16 years since commercial introduction. While most of the pesticide use data incorporated in the model were originally reported by U.S. government agencies in pounds of active ingredient, and/or pounds of a.i./acre, results are reported herein in SI units (kilograms of active ingredient and kg/ha). Some key results are also reported in pounds/acre. Convert kilograms to pounds by multiplying by 2.205, and pounds to kgs by multiplying by 0.454. To convert from kg/ha to pounds/acre, multiply by 0.893; to convert from pounds/acre to kg/ha, multiply by 1.12.

Results and discussion

Farmers planted 0.55 billion hectares (1.37 billion acres) of HR corn, soybeans, and cotton from 1996 through 2011, with HR soybeans accounting for 60% of these hectares [Additional file 1: Table S7]. In terms of overall herbicide use per hectare based on NASS data, substantial increases have occurred from 1996 through 2011. In soybeans, USDA reported herbicide applications totaling 1.3 kgs/ha (1.17 pounds/acre) in 1996, and 1.6 kgs/ha (1.42 pounds/acre) in 2006, the last year soybeans were surveyed by USDA. In cotton, herbicide use has risen from 2.1 kgs/ha (1.88 pounds/acre) in 1996 to 3.0 kgs/ha (2.69 pounds/acre) in 2010, the year of the most recent USDA survey. In the case of corn, herbicide use has fallen marginally from 3.0 kgs/ha (2.66 pounds/acre) in 1996 to 2.5 kgs/ha

(2.26 pounds/acre) in 2010, largely as a result of lessened reliance on older, high-rate herbicides.

Compared to herbicide use rates per hectare on non-HR hectares, HR crops increased herbicide use in the U.S. by an estimated 239 million kgs (527 million pounds) in the 1996–2011 period, with HR soybeans accounting for 70% of the total increase across the three HR crops. Rising reliance on glyphosate accounted for most of this increase.

In light of its generally favorable environmental and toxicological properties, especially compared to some of the herbicides displaced by glyphosate, the dramatic increase in glyphosate use has likely not markedly increased human health risks. Because glyphosate cannot be sprayed on most actively growing, non-GE plants, residues of glyphosate in food have been rare, at least until the expansion ~ 2006 in the number of late-season glyphosate applications on wheat and barley as a harvest aid and/or to control escaped weeds. Presumably as a result of such uses, 5.6% of 107 bread samples tested in 2010 by the U.K. Food Standards Agency contained glyphosate residues [9]. Three samples had 0.5 parts per million of glyphosate [9], a relatively high level compared to the other pesticides found in these bread samples.

Budget pressures have forced the U.S. Department of Agriculture to reduce the number of crops included in its annual NASS pesticide use survey. Soybean pesticide use has not been surveyed since 2006, about when the spread of glyphosate-resistant weeds began to significantly increase herbicide use in selected areas. Herein, total herbicide use on HR hectares is projected to rise 13.5% from 2006–2011 (about 2.7% annually), compared to a 6.6% (1.3% annually) increase on conventional soybean hectares. By way of contrast, the NASS-reported glyphosate rate of application per crop year on the average hectare of soybeans increased 8.9% per annum from 2000–2006 (see Table 1). So, despite the significant and widespread challenges inherent in managing glyphosate-resistant weeds in the 2006–2011 period, a substantial decrease is projected in the rate of increase in glyphosate applications per hectare of HR soybeans. The justification for this projected fall in the rate of increase is recognition by farmers that further increases in glyphosate use will likely not prove cost-effective, coupled with positive responses by farmers to the near-universal recommendation that corn-soybean farmers incorporate into their spray programs herbicides that work through modes of action other than glyphosate's [10-15].

Since 1996, about 317 million trait hectares (782 million trait acres) have been planted to the three major *Bt* traits – *Bt* corn for European corn borer (ECB) and CRW, and *Bt* cotton. *Bt* corn and cotton have delivered consistent reductions in insecticide applications totaling 56 million kgs (123 million pounds) over 16 years of commercial use. *Bt* corn reduced insecticide use by 41 million kgs (90 million pounds), while *Bt* cotton displaced 15 million kgs (34 million pounds) of insecticide use.

Table 1 Projected rates of change in herbicide use since the most recent USDA survey, relative to recent annual percent changes in rates

	2010-2011	2005-2010	Per Year 2005-2010
Corn			
Total Herbicides	2%	10.2%	2.0%
Glyphosate	2.5%	12.9%	2.6%
Soybeans	**2007-2011**	**2000-2006**	**Per Year 2000-2006**
Total Herbicides	3.2%	35.2%	5.9%
Glyphosate	3.3%	53.4%	8.9%
Cotton	**2010-2011**	**2007-2010**	**Per Year 2007-2010**
Total Herbicides	2.2%	3.1%	1.0%
Glyphosate	-1%	-10.3%	-3.4%

Taking into account applications of all pesticides targeted by the traits embedded in the three major GE crops, pesticide use in the U.S. was reduced in each of the first six years of commercial use (1996–2001). But in 2002, herbicide use on HR soybeans increased 8.6 million kgs (19 million pounds), driven by a 0.2 kgs/ha (0.18 pounds/acre), increase in the glyphosate rate per crop year, a 21% increase. Overall in 2002, GE traits increased pesticide use by 6.9 million kgs (15.2 million pounds), or by about 5%. Incrementally greater annual increases in the kilograms/pounds of herbicides applied to HR hectares have continued nearly every year since, leading to progressively larger annual increases in overall pesticide use on GE hectares/acres compared to non-GE hectares/acres. The increase just in 2011 was 35.3 million kgs (77.9 million pounds), a quantity exceeding by a wide margin the cumulative, total 14 million kg (31 million pound) reduction from 1996 through 2002.

Total pesticide use has been driven upward by 183 million kgs (404 million pounds) in the U.S. since 1996 by GE crops, compared to what pesticide use would likely have been in the absence of HR and *Bt* cultivars. This increase represents, on average, an additional ~0.21 kgs/ha (~0.19 pounds/acre) of pesticide active ingredient for every GE-trait hectare planted. The estimated overall increase of 183 million kgs (404 million pounds) applied over the past 16 years represents about a 7% increase in total pesticide use.

There are two major factors driving the upward trend in herbicide use on HR hectares compared to hectares planted to non-HR crops: incremental reductions in the application rate of herbicides other than glyphosate applied on non-HR crop hectares, and second, the emergence and rapid spread of glyphosate-resistant weeds. The first factor is driven by progress made by the

pesticide industry in discovering more potent herbicidal active ingredients effective at progressively lower rates of application.

Twenty-seven percent of U.S. soybean hectares in 1996 were treated with pendimethalin at an average rate of 1.1 kgs/ha and another 22% were sprayed with trifluralin at a rate of 0.99 kgs/ha, while the market leader (imazethapyr) was applied to 43% of hectares planted at a rate of 0.07 kgs/ha [16]. By 2002 the combined percentage of soybean hectares treated with these two high-dose herbicides had dropped from 49% to 16% [17], and just 5% were treated in 2006 [18]. Between 1996 and 2006, the number of registered soybean herbicides applied at rates below 0.11 kgs/ha increased from nine to 17. As a result, the amount of herbicides applied to conventional crops has steadily fallen since 1996. In contrast, glyphosate is a relatively high-dose herbicide that is usually applied at a rate between 0.67 to 0.9 kgs per hectare.

Resistant weeds

The emergence and spread of glyphosate-resistant weeds is the second, and by far most important factor driving up herbicide use on land planted to herbicide-resistant varieties. Glyphosate resistant (GR) weeds were practically unknown before the introduction of RR crops in 1996. The first glyphosate-resistant weed (*Lolium rigidum*) emerged in Australia in 1996 from canola, cereal crop, and fence line applications [19]. In the mid-1990s, as the first glyphosate-resistant crops were moving toward commercialization and gaining market share, Monsanto scientists wrote or were co-authors on several papers arguing that the evolution of GR weeds was unlikely, citing the herbicide's long history of use (~20 years) and relative absence of resistant weeds [20,21].

Other scientists, however, challenged this assertion [22]. Dr. Ian Heap, long-time manager of the international database on resistant weeds, warned in a 1997 conference presentation that to limit glyphosate selection pressure in Roundup Ready cropping systems, the herbicide would need to be used in conjunction with proven resistance-management practices and with non-chemical weed control methods [23]. A 1996 report by Consumers Union stated that HR crops are "custom-made" for accelerating resistance and called for the Environmental Protection Agency (EPA) to revoke approval of HR crops when and where credible evidence of resistance emerges [24].

Today, the Weed Science Society of America (WSSA) website lists 22 GR weed species in the U.S. [19]. Over two-thirds of the approximate 70 state-GR weed combinations listed by WSSA have been documented since 2005, reflecting the rapidly spreading nature of the GR-weed problem. According to the WSSA, over 5.7 million hectares (14 million acres) are now infested by GR weeds, an estimate that substantially underestimates the actual spread of resistant weeds [16,22], [and personal communication, Dr. Ian Heap]. Dow AgroSciences carried out a recent survey on the percent of crop acres/hectares in the U.S. impacted by glyphosate-resistant weeds [25]. Findings from the survey were provided to USDA in support of Dow AgroSciences's petition for deregulation of 2,4-D herbicide-resistant corn, and suggest that around 40 million hectares (100 million acres) are already impacted by glyphosate-resistant weeds, an estimate that Heap considers inflated [personal communication]. The true extent of spread in the U.S. likely lies around the midpoint between the WSSA and Dow AgroSciences estimates (i.e., 20–25 million hectares), and by all accounts, will continue to rise rapidly for several years.

Why have GR weeds become such a serious problem? Heavy reliance on a single herbicide – glyphosate (Roundup) – has placed weed populations under progressively intense, and indeed unprecedented, selection pressure [10]. HR crops make it possible to extend the glyphosate application window to most of the growing season, instead of just the pre-plant and post-harvest periods. HR technology allows multiple applications of glyphosate in the same crop year. The common Midwestern rotation of HR corn-HR soybeans, or HR soybeans-HR cotton in the South, exposes weed populations to annual and repetitive glyphosate-selection pressure.

These factors trigger a perfect storm for the emergence of GR weeds. Research has traced the resistance mechanism in Palmer amaranth (*Amaranthus palmeri*) to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene amplification. Resistant weed populations from Georgia contained 5-fold to 160-fold more copies of the EPSPS gene, compared to susceptible plants [26]. Moreover, EPSPS gene amplification is heritable, leading Gaines et al. to warn that the emergence of GR weeds "endangers the continued success of transgenic glyphosate-resistant crops and the sustainability of glyphosate as the world's most important herbicide."

Resistant Palmer amaranth (*Amaranthus palmeri*) has spread dramatically across southern states since the first resistant populations were confirmed in 2005, and already poses a major economic threat to U.S. cotton production. Some infestations are so severe that cotton farmers have been forced to leave some crops unharvested.

Responding to resistance

GR weed phenotypes are forcing farmers to respond by increasing herbicide application rates, making multiple applications of herbicides, applying additional herbicide active ingredients, deep tillage to bury weed seeds, and manual weeding. In recent years the first three of the above responses have been the most common. Each response increases the kilograms of herbicides applied on HR crop hectares. All five interventions increase costs.

Moreover, if 2,4-D and dicamba herbicide-resistant corn and soybeans are fully deregulated by the U.S. government, there will be growing reliance on older, higher-risk herbicides for management of glyphosate-resistant weeds.

Based on an upward trajectory in the planting of 2,4-D HR corn reaching 55% of corn hectares planted by 2019, coupled with an average of 2.3 applications (the label allows three) and an average rate of 0.94 kgs/ha (0.84 pounds/acre) (the label allows 1.12 kgs/ha (1.0 pounds/acre)), 2,4-D use on corn in the U.S. would increase over 30-fold from 2010 levels [Additional file 1: Table S19]. Such a dramatic increase could pose heightened risk of birth defects [27,28] and other reproductive problems [29], more severe impacts on aquatic ecosystems [30], and more frequent instances of off-target movement and damage to nearby crops and plants. Moreover, the efficacy of 2,4-D corn may well prove short lived, since a population of 2,4-D resistant waterhemp (*Amaranthus tuberculatus*) has now been confirmed in Nebraska [31], and there are already at least eight other weeds resistant to 2,4-D [19].

GR weeds typically emerge first on a few isolated fields, but their pollen, genes, and seeds can travel widely and spread quickly, especially if glyphosate continues to be relied on heavily [11]. No substantial change in the intensity of glyphosate use in the U.S. is expected in the foreseeable future; nearly all corn, soybean, and cotton cultivars now carry a RR gene. The seed industry has no plans to grow and sell more non-HR seed, and indeed is moving in the opposite direction by developing more stacked, multiple HR varieties. The share of total national corn, soybean, and cotton hectares impacted by GR weed populations is likely to grow and will, as a result, increase both the number of different herbicides applied, as well as the total kgs of herbicides applied.

As argued by many weed scientists and extension specialists, integrated weed management systems, coupled with markedly lessened reliance on RR technology are now essential to extend the useful life of RR technology [10,12,14,32]. Without major change, a crisis in weed management systems is likely, triggering possibly ominous economic, public health, and environment consequences.

Higher costs triggered by resistant weeds and HR technology

Weed management costs per hectare increase by 50% to 100% or more in fields infested with glyphosate-resistant weeds, as evident in a series of case studies submitted to the USDA by Dow AgroSciences in support of its petition to the USDA seeking deregulation of 2,4-D herbicide-resistant corn [25]. In soybean production in Arkansas, for example, Dow AgroSciences compared the average cost/acre of the top-five, most popular herbicide programs in Roundup Ready soybeans in fields without resistant weeds, compared to the average of two recommended programs in fields infested with glyphosate-resistant Palmer amaranth. Herbicide costs rise 2.7-fold (from $16.29 to $44.34 per acre) [23], [Table thirty, page 93]. In Illinois soybean production, the increase in herbicide costs is estimated at 64% ($19.21 to $31.49 per acre) [23], [Table thirty-two, page 95], while in Iowa corn production, the increase is 67% ($19.23 to $32.10 per acre) [23], [Table thirty-six, page 99].

The markedly higher cost/hectare of herbicide-resistant seeds must be added to the higher herbicide costs noted above to more fully reflect the added costs associated with HR technology. The cost of a bushel of conventional, not-GE soybean seed increased during the GE-crop era from $14.80 in 1996 to $33.70 in 2010, while a bushel of GE soybean seed cost, on average, $49.60 in 2010 (all seed price data derived from USDA data) [33]. Accordingly, the cost of GE soybean seed in 2010 was 47% higher per bushel than non-GE seed. In the case of corn, conventional seed prices rose from $26.65 per acre planted in 1996 to $58.13 in 2010. The average cost of GE corn seed per acre in 2010 was $108.50, with some GE cultivars selling for over $120 per planted acre. Hence, GE corn seed costs per acre were about double the cost conventional seed.

Public health concerns

Heightened risk of public health impacts can be expected in the wake of more intensive herbicide use, especially applications later in the season on herbicide-resistant crop varieties. While current risk assessment science suggests that glyphosate is among the safer herbicides per hectare treated in terms of human health risks, both the frequency of human exposures and levels of exposure via food, drinking water, and the air have no doubt risen in the U.S. in recent years. Two-thirds to 100% of air and rainfall samples tested in Mississippi and Iowa in 2007–2008 contained glyphosate [34].

The likely approval and use of herbicide-resistant crops in the U.S. engineered to survive applications of multiple herbicides adds tricky new dimensions to herbicide-risk assessments. Applications later in the growing season will be more likely to lead to residues in silage or forage crops. As a result, herbicide residues in milk, meat, or other animal products might become more common. The jump in herbicide volumes applied during June and July will increase the risk of drift and herbicide movement via volatilization, possibly exposing people via the air, water, or crops grown in the proximity of treated fields. Risks from the drift and volatilization of 2,4-D and dicamba are of special concern, given that these two herbicides have triggered thousands of non-target crop damage episodes over the last 20 years in the U.S. Indeed, for several years,

2,4-D has been the leading cause of crop damage episodes investigated by State departments of agriculture [35].

Environmental impacts linked to HR technology

A long list of environmental effects can be triggered, or made worse, by the more intensive herbicide use required to keep pace with weeds in farming systems heavily reliant on herbicide-resistant crops. Glyphosate has been shown to impair soil microbial communities in ways that can increase plant vulnerability to pathogens [36-38], while also reducing availability of certain soil minerals and micronutrients [39]. Landscapes dominated by herbicide-resistant crops support fewer insect and bird species; e.g., a study in the American Midwest reported a 58% decline in milkweed and an 81% drop in monarch butterflies from 1999 to 2010 [40]. Heavy use of glyphosate can reduce earthworm viability [41] and water use efficiency [42]. Several studies have documented reductions in nitrogen fixation in herbicide-resistant soybean fields sprayed with glyphosate [43,44]. Transgene flow from herbicide-resistant crops can occur via multiple mechanisms and can persist in weedy relatives [45].

Individually, these environmental impacts appear, for the most part, of the same nature and in the same ballpark as the risks associated with other herbicide-based farming systems, but collectively they raise novel concerns over long-term, possibly serious impacts on biodiversity, soil and plant health, water quality, aquatic ecosystem integrity, and human and animal health.

Bt corn and cotton impacts and prospects

While *Bt*-transgenic corn and cotton have displaced an estimated 56 million kgs (123 million pounds) of insecticides since 1996, every plant in a *Bt* corn or cotton field is manufacturing within its cells one or more forms of the natural bioinsecticide *Bacillus thuringiensis*. The rate of synthesis of *Bt* Cry protein endotoxins is roughly proportional to the rate of plant growth. As plants mature and enter senescence, *Bt* endotoxin expression falls.

Few published estimates are available of *Bt* endotoxin expression levels in contemporary corn cultivars. Nguyen et al. projected that a hectare of *Bt*-corn for CRW control expressing the Cry3Bb1 gene in MON88017 corn produces 905 grams of Cry3Bb1 per hectare (0.8 pounds per acre) [46]. The amount of *Bt* Cry proteins produced by a hectare of *Bt* corn for ECB and CRW control are calculated in [Additional file 1: Tables S20–S22], with key results shown in Table 2 for specific corn events, traits, and endotoxins. [Additional file 1 Tables S23–25] cover *Bt* cotton events. Expression level data reported by companies in regulatory documents were used to calculate per hectare production of specific endotoxins. [Additional file 1: Tables S21 and Table S24 contain the expression level data for *Bt* corn and cotton events, and [Additional file 1: Table S22 and Table S25] report the volumes of *Bt* Cry proteins produced per hectare and acre based on contemporary seeding rates.

Major contemporary *Bt* corn events targeting the ECB synthesize nearly as much or more insecticidal Cry protein per hectare than the weighted-average rate of conventional

Table 2 *Bt* cry protein synthesis in major GE corn cultivars

	Cry Protein	Cry/Shoot	Cry/Root	Cry/Plant	Plants per hectare	Cry Toxin kg /ha	Plants per Acre	Cry Toxin lb/acre
MON 810	Cry1Ab	1193	496	1689	79,040	0.133	32,000	0.119
MON 88017	Cry3Bb1	14915	4030	18945	79,040	1.497	32,000	1.333
MON 89034	Cry1A.105	2826	620	3446	79,040	0.272	32,000	0.242
MON 89034	Cry2Ab2	4553	496	5049	79,040	0.399	32,000	0.355
TC 1507	Cry1F	1207	165	1372	79,040	0.108	32,000	0.097
DAS 59122	Cry34Ab1	26376	2647	29023	79,040	2.294	32,000	2.042
DAS 59122	Cr35Ab1	5825	567	6392	79,040	0.505	32,000	0.45
SmartStax Corn								
MON 88017	Cry3Bb1	7536	2015	9551	79,040	0.755	32,000	0.672
MON 89034	Cry1A.105	2983	651	3634	79,040	0.287	32,000	0.256
MON 89034	Cry2Ab2	4553	558	5111	79,040	0.404	32,000	0.36
TC 1507	Cry1F	1413	185	1598	79,040	0.126	32,000	0.112
DAS 59122	Cry34Ab1	24649	2623	27272	79,040	2.156	32,000	1.918
DAS 59122	Cr35Ab1	5275	586	5861	79,040	0.463	32,000	0.412
SmartStax Total						**4.191**		**3.73**

insecticides applied on a hectare planted to *Bt* corn for ECB control (about 0.15 kgs insecticide per ha; 0.13 pounds/acre in 2010 [Additional file 1: Table S11]). MON810, the Cry protein in Monsanto's original Yieldgard corn, expresses 0.2 kgs/ha of endotoxin, whereas Syngenta's *Bt* 11 synthesizes 0.28 kgs/ha [Additional file 1: Table S22]. Newer events for ECB control like Monsanto's Genuity VT Double PRO (MON 89034) produce Cry 1A.105 and Cry 2Ab2 endotoxins totaling 0.62 kgs/ha. The Dow AgroSciences-Pioneer Hi-Bred Herculex I (TC1507) event expresses the least endotoxin – 0.1 kg *Bt* endotoxin per hectare – just below the rate of insecticides applied.

In the case of *Bt* corn targeting the CRW, every hectare planted in recent years expresses substantially greater volumes of *Bt* endotoxins than the ~0.2 kgs of insecticides applied on the average hectare for CRW control (0.19 pounds/acre [Additional file 1: Table S12]). MON 88017 expresses 0.62 kgs/ha of Cry 3Bb1, while DAS 59122–7 expresses two Cry proteins totaling 2.8 kgs/ha, 14-fold more than the insecticides displaced [Additional file 1: Table S22]. SmartStax GE corn synthesizes six Cry proteins, three targeting the ECB, and three the CRW. Total Cry protein production is estimated at 4.2 kgs/ha (3.7 pounds/acre), 19-times the average conventional insecticide rate of application in 2010.

Should Bt endotoxins count as insecticides applied?

Entomologists are divided on the question of whether the *Bt* produced by transgenic plants should be counted as "insecticides applied." The case for doing so is strong, despite the obvious differences in how Cry proteins enter corn agroecosystems. When a field of corn is sprayed with a foliar *Bt* insecticide, the amount of toxin sprayed per hectare should be counted when computing total insecticide use. The primary difference between the *Bt* Cry proteins in a *Bt*-transgenic plant, and a field of non-GE plants sprayed with foliar *Bt*, is that in the later case, the toxin is present predominantly on plant tissue surfaces, whereas in the former *Bt*-crop case, the toxin is inside plant cells. This distinction does not greatly matter from the perspective of the overall load of pesticides in the environment, although the presence of pesticides inside plants, as opposed to on their surface, alters relative risk profiles across non-target organisms.

It should also be noted that, in general, the systemic delivery of *Bt* Cry proteins poses more significant risks to animals and humans ingesting *Bt* crops than applications of *Bt* insecticides via liquid sprays. Systemic delivery also enhances a range of environmental and ecological risks [47] compared to foliar *Bt* use patterns that result in rapid breakdown of *Bt* Cry proteins as they are exposed to sunlight and rainfall.

Most corn insecticides are applied in ways that expose active ingredients to destructive abiotic and biotic forces that tend to break down the chemicals to generally less toxic forms. Granular soil insecticides applied via boxes on corn planters tend to break down within weeks as a result of soil microbial activity. Because properly applied granular insecticides are buried in the soil, exposure to non-target organisms is limited, although poorly operated or calibrated planting equipment can result in grains of insecticide remaining on the soil surface, posing a serious potential risk to some bird species. A significant portion of the foliar insecticides applied per hectare for ECB control never hit its plant target, and a portion of the insecticide that does land and lodge on plant tissues is washed off within hours, days, or weeks during rainfall events. This is why insecticide residues are rarely detected in corn grain and silage at harvest time, and why conventional insecticide applications on corn pose little or no human dietary risk.

By virtue of their altered environmental fate and risk profile, all systemic pesticides should be counted when measuring pesticide use, and hence so too should the *Bt* proteins manufactured in *Bt*-transgenic crops. If *Bt*-transgenic plants produced proteins that disrupted insect morphology, feeding behavior, or reproduction, the absence of a toxic mode of action would strengthen the argument that *Bt* Cry proteins are not functionally equivalent to insecticides, and hence should not be counted as insecticides applied. *Bt*-crop technology that limits *Bt*-endotoxin expression to only those tissues that are under active attack, and then only during times when insects are actively feeding, would also support the view that *Bt* crops are compatible with IPM.

Conclusions

Today's pest-management related GE traits have proven popular and commercially profitable for the biotech-seed industry, but over-reliance has set the stage for resistance-driven problems in both herbicide-resistant and *Bt*-transgenic crops. Largely because of the spread of glyphosate-resistant weeds, HR crop technology has led to a 239 million kg (527 million pound) increase in herbicide use across the three major GE-HR crops, compared to what herbicide use would likely have been in the absence of HR crops. Well-documented increases in glyphosate applications per hectare of HR crop account for the majority of this 239 million kg increase.

While *Bt* corn and cotton have reduced insecticide applications by 56 million kgs (123 million pounds), resistance is emerging in key target insects and substantial volumes of *Bt* Cry endotoxins are produced per hectare planted [corn, Additional file 1: Tables S20–S22, cotton, Additional file 1: Tables S23–S25], generally dwarfing the volumes of insecticides displaced. Documenting the

full range of impacts on the environment and public health associated with the *Bt* Cry proteins biosynthesized inside *Bt*-transgenic plants remains a challenging and largely ignored task, especially given the recent move toward multiple *Bt* protein, stacked-trait events.

Overall, since the introduction of GE crops, the six major GE technologies have increased pesticide use by an estimated 183 million kgs (404 million pounds), or about 7%. The spread of GR weeds is bound to trigger further increases, e.g., the volume of 2,4-D sprayed on corn could increase 2.2 kgs/ha by 2019 (1.9 pounds/acre) if the USDA approves unrestricted planting of 2,4-D HR corn [Additional file 1: Table S19]. The increase in herbicides applied on HR hectares has dwarfed the reduction in insecticide use over the 16 years, and will almost surely continue to do so for several more years.

Estimating the impacts of GE crops on pesticide use is growing more complex because of gaps in NASS pesticide use data collection for the three major crops, increases in the average number of traits per GE-crop hectare planted, the registration of HR crops engineered to resist herbicides other than glyphosate, massive disruption in weed communities, and the presence of one to three, or even more, glyphosate-resistant weeds in many crop fields. It is difficult to project what the distribution, population levels, and phenotypes of weeds would have been over the last 16 years in the absence of HR technology. Inevitably, weed management systems and technology would have evolved along other trajectories in the absence of HR crops these last 16 years, resulting in heightened reliance on both preplant and post-emergence applications of multiple, low-dose herbicides.

A majority of American soybean, maize, and cotton farmers are either on, or perilously close to a costly herbicide and insecticide treadmill. Farmers lack options and may soon be advised, out of necessity, to purchase HR crop cultivars resistant to multiple active ingredients and to treat *Bt* corn with once-displaced corn insecticides. The seed-pesticide industry is enjoying record sales and profits, and the spread of resistant weeds and insects opens up new profit opportunities in the context of the seed industry's current business model. Regulators cannot restrict the use of a previously approved HR technology because it increases pesticide use and triggers resistance, nor have U.S. government agencies turned down an application for a new HR or *Bt*-transgenic trait because of the likelihood it would accelerate the spread of resistant weeds or insects. Whether the USDA has the statutory authority to deny a petition for HR crop deregulation (i.e., approval) on the grounds of worsening problems with resistant weeds is a contested issue in ongoing litigation.

Profound weed management system changes will be necessary in the three major GE crops to first stabilize, and then hopefully reduce herbicide use, the costs of weed management, and herbicide-related impacts on human health and the environment. Weed management experts are largely in agreement that the percent of cropland area planted to glyphosate-based HR seeds must decline dramatically (e.g., by at least one-third to one-half) for farmers to have a realistic chance at success in preventing resistance [10,12,14]. Unfortunately, there appears little interest across the seed-biotech industry in increasing production of non-Roundup Ready or not-*Bt* transgenic seed. Since the decisions made by the seed industry in any given year determine the traits offered by the industry to farmers in next crop season, the seed industry must act first in order for farmers to turn the corner toward more sustainable weed and insect pest management systems. The many important ramifications of this practical reality – that the seed industry must act first – have yet to be fully appreciated by farmers, weed management experts, and policy makers in the U.S.

Regulators in the U.S. have thus far done little to prevent the emergence and spread of resistant weeds, while several resistance-management interventions have been imposed as part of the approval of *Bt* crops. In addressing weed resistance, the hands-off regulatory posture in the U.S. reflects, in part, the basic authorities granted to the EPA and USDA in federal law. Both agencies regard weed resistance as an efficacy-economics challenge that can best be addressed by the private sector consistent with market forces. The need for novel policy interventions will grow in step with the emergence and spread of resistance weeds and evidence of adverse economic, environmental, and public health consequences triggered by markedly increasing reliance on older, higher-risk herbicides.

Methods

The model calculates the impact of HR and *Bt*-transgenic crop varieties on pesticide use annually from 1996 through 2011, and aggregates results over this 16-year period. The model is composed of 16 tables [Additional file 1: Tables S1–S16]. Nine additional tables, [Additional file 1: Tables S17–S25] address changes in pesticide use, the spread of resistant weeds, and the quantity of *Bt* endotoxins produced per hectare by today's major corn and cotton *Bt* traits.

The model was developed using the units of measure typical in USDA-NASS surveys (pounds of active ingredients, acres planted); the Additional files are available in pounds and acres units only. In this paper, metric units are used to report results, although selected key results will be reported in both units of measure.

[Additional file 1: Table S1] records average per acre herbicide and insecticide use data, drawing on pesticide use data compiled annually by the USDA's NASS. These surveys record the percent of crop acres treated with

specific active ingredients, average one-time rates of application, the average number of applications, the rate per crop year (average rate multiplied by the average number of applications), and total pounds applied.

In the case of herbicides, [Additional file 1: Table S1] reports total herbicide, all glyphosate, and "Total Herbicides Minus Glyphosate." "All Glyphosate" aggregates the multiple chemical forms of glyphosate surveyed by NASS, and calculates average rates of application and number of applications, weighted by frequency of use. The same procedure is used to calculate average pounds/acre applied of other herbicides of interest for which NASS reports use data for multiple chemical forms (e.g. 2,4-D, dicamba). [Additional file 1: Table S2] includes national acres planted to each crop, average pesticide use rates, and total pounds applied per acre and overall herbicide, insecticide, and herbicide + insecticide volumes applied.

[Additional file 1: Tables S3–S6] record the percent of national acres planted to a crop variety expressing each of the six, major commercial GE traits. The USDA's ERS provides data on the percent of total national corn [Additional file 1: Table S3], soybean [Additional file 1: Table S4], and cotton hectares [Additional file 1: Table S5] that were planted to each GE crop trait for 1996–2011. Percent acres planted to all six GE traits by year are presented in [Additional file 1: Table S6]; there is a high level of confidence in these data.

[Additional file 1: Table S7] reports acres planted to each of the six traits, multiplying the percent acres planted to each trait in ST 6 by total acres planted to each crop in [Additional file 1: Table S2]. [Additional file 1: Tables S8–S10] calculate, for the three HR crops, the estimated difference in average herbicide use on HR hectares versus land planted to conventional, non-GE varieties. [Additional file 1: Tables S11–S13] report the basis for calculating the pounds of insecticides displaced by the planting of *Bt* corn and cotton traits. [Additional file 1: Table S14] integrates all of the average per acre pesticide use rates by crop, trait and year, and reports the estimated difference between per acre rates on GE versus non-GE acres. [Additional file 1: Table S15] converts the differences in rates per acre to differences in pounds applied nationally by crop, trait, and year, and over the 16-year period. [Additional file 1: Table S16] provides details on glyphosate use from NASS surveys over the 1996–2010 period, and is the source of data on glyphosate use in other Additional files.

Assumptions, projections, and calculations

A series of assumptions, projections, and calculations are embedded in the model in order to estimate total herbicide and insecticide use on GE versus not-GE hectares. Table 3 outlines model assumptions and Table 4 describes the projections embedded in the model's calculations.

NASS surveyed corn, soybean, and cotton pesticide use in most years from 1996–2010. None of the crops were surveyed in 2008; cotton was last surveyed in 2007 and 2010; corn was surveyed in 2005 and 2010; and soybeans have not been surveyed since 2006. In estimating the impacts of GE crops on pesticide use from 1996–2011, average application rates per crop year were interpolated in years with no data, when NASS had surveyed a previous and subsequent year, based on the assumption of linear change in the intervening years.

It is assumed that changes in the volume of herbicides other than glyphosate applied on the average HR hectare tracks changes in total herbicide use, and also changes gradually from year-to-year. With few exceptions, these

Table 3 Data sources and assumptions required to quantify the impact of GE crops on pesticide use in the U.S., 1996-2011

Parameter	Source	Supplemental table impacted	Basis and explanation
National Pesticide Use per Acre/Hectare	NASS-USDA	1, 2	Best publicly available estimates of annual per acre herbicide and insecticide use
Annual Gaps in NASS Survey Data by Crop	Interpolated	1, 2	Changes in total herbicide, glyphosate, and insecticide use occur linearly/annum when there are gaps in NASS pesticide use surveys
Annual Application Rates of "Other Herbicides on HR Hectares"	(See Table 4)	8, 9, 10	Trends by crop on HR acres track changes in total herbicide use, as reported by NASS; changes from year to year are gradual
Bt Cry Proteins Produced by *Bt* Corn and Cotton Plants	Projected (see text, Additional files)	20-25	Trait-specific expression levels by tissue taken from documents submitted by technology developers; used to quantify volume of each *Bt* endotoxin produced by plants per acre/hectare based on typical planting density
Insecticide Use on *Bt* Corn	(Details in Table 4)	11, 12	Insecticide displacement as a result of planting *Bt* corn corrected for hectares not likely to have been treated in the absence of *Bt* corn cultivars
Insecticide Use on *Bt* Cotton	NASS-USDA	13	Budworm/bollworm control insecticide displacement on hectares planted to *Bt* cotton is 100%

patterns of change in herbicide use are evident in all crops surveyed by USDA. Significant annual changes in total herbicide use, as well as non-glyphosate applications, are almost always linked to an increase or decrease in acres treated with one or more relatively high-dose herbicides applied at or around 1 pound/acre, compared to use of herbicides applied at rates less than 0.5 pound/acre (several are sprayed at rates below 0.05 pounds/acre).

The volumes of *Bt* Cry endotoxins produced per acre/hectare of *Bt* corn and cotton are not included in the estimates of changes in insecticide use on acres/hectares planted to *Bt* cultivars, although the volumes are surprisingly significant compared to the volume of insecticides applied on treated acres/hectares (see "Discussion"). In the case of insecticide use on *Bt* corn, the volume of insecticide use displaced per acre/hectare is adjusted in light of the likely percent of *Bt* corn acres/hectares that would have been treated with an insecticide in the absence of *Bt* cultivars. Multiple analysts have reported substantial planting of *Bt* corn as insurance against possible insect feeding damage, on acres/hectares that farmers would not prophylactically apply insecticides [4,13]. In a January 2010 survey, 73.3% of 518 farmers surveyed at regional extension meetings in Illinois reported that they planted *Bt* corn "Knowing That Anticipated Damage Levels Were Low" [48]. USDA has surveyed corn insecticide use 14 times since 1991. The total area treated with an insecticide has fallen in the range 31% +/− 5% in all years, with the average around 33%.

It is assumed that farmers planting *Bt* cotton do not spray conventional insecticides against the budworm/bollworm complex of insecticides, leading to 100% displacement of such applications. This assumption likely overestimates displacement marginally, especially in recent years where isolated populations of less susceptible or resistant populations have emerged.

Table 3 describes the basis for projecting a number of missing values over the 1996-2011-time period. In the years since the last NASS survey, pesticide rates were projected based on recent trends and changes in weed pressure.

In the case of corn, total herbicide and glyphosate use trends from 2005–2010 are projected to continue unchanged through 2011, despite the accelerating emergence and spread of resistant weeds in the Midwest. The rapid rate of increase in total herbicide and glyphosate use/acre in soybean production systems from 2000–2006 (5.9% and 8.9%/annum) is projected to decline to an average increase of 3.2% and 3.3% per annum in 2007–2011. Reductions in annual rates of increase reflect the decision by many HR soybean farmers to follow the advice of weed management specialists [10,11] to diversity the modes of action included in herbicide-based control programs. The rate of increase in total herbicide use on HR cotton from 2010 to 2011 is projected at about twice the annual rate, 2007–2010, whereas the rate of decline in per hectare glyphosate use is projected to fall from −3.4% to −1% per annum as farmers increase rates and/or frequency of applications of glyphosate in regions where resistant weeds are now posing serious management challenges.

Estimating herbicide use on conventional and HR hectares

NASS surveys do not report pesticide use on GE and conventional crop hectares separately.

The volume of herbicides applied to HR hectares can be approximated by adding NASS-reported glyphosate use

Table 4 Projections required quantifying the impact of GE crops on insecticide use in the US, 1996–2011

Parameter	Supplemental table(s) impacted	Basis for setting value	Basis and explanation
Corn			
Share of Insecticide Applications Targeting the European Corn Borer (ECB) Versus Corn Rootworm (CRW)	11, 12	Guidance from extension IPM specialists and land grant university spray guides	Some insecticides applied exclusively for control of ECB, others for control of CRW; and some target both. The percent hectares treated with a given insecticide are apportioned relative to target pests: ECB, CRW, or other insects.
"Other Insecticides" Applied in 2010 for ECB Control	11	NASS data on "Other Insecticides" applied in 2010	NASS reported 237,000 pounds of "Other Insecticide" use in 2010; 30% of these "Other Insecticides" applied to corn in 2010 projected to target the ECB.
"Other Insecticides" Applied in 2010 for CRW Control	12	NASS data on "Other Insecticides" applied in 2010	NASS reported 237,000 pounds of "Other Insecticide" use in 2010; 60% of "Other Insecticides" applied to corn in 2010 projected to target the CRW.
Cotton			
Share of Insecticide Applications Targeting the Budworm/Bollworm Complex	13	Guidance from extension IPM specialists and land grant university spray guides	Some insecticides applied exclusively or partly for control of the budworm/bollworm complex, others for other insects; percent hectares treated with a given insecticide is apportioned relative to target insects.

per crop year to an estimate of the volume of herbicides other than glyphosate (hereafter, "other herbicides") applied on HR hectares. The volume of "other herbicides" applied on HR hectares is estimated based on the average number of non-glyphosate herbicides applied per hectare, coupled with the average rate per application of non-glyphosate herbicides. In addition, the rate of "other herbicides" on HR hectares is adjusted to reflect changes from year to year in overall herbicide use and glyphosate application rates. For example in recent years, "other herbicides" have been applied to around one-half of HR soybean hectares at an average rate of ~0.34 kgs/ha (~0.3 pounds/acre), resulting in an average ~0.17 kgs/ha (~0.15 pounds/acre) of "other herbicide" applications on all HR hectares (0.5 × 0.34).

The shares of total crop hectares in a given year planted to conventional and HR crop varieties is compiled by the USDA's ERS [Additional file 1: Tables S3–S5] and can be used in a weighted-average formula to calculate the kgs of herbicides applied on non-HR hectares –

$$THA\ \ Crop_x = [(\%HPHT_x)x(HAHT_x)] + [(\%HPCON_x)x(HACON_x)]$$

Where,

THA $Crop_x$ = "Total Herbicides Applied" (kgs active ingredient/hectare in a crop year);

% $HPHT_x$ = Percent national "Hectares Planted to HR" cultivars;

$HAHT_x$ = "Herbicides Applied on HR" hectares (kg a.i./crop year);

% $HPCON_x$ = Percent national "Hectares Planted to Conventional" non-HR hectares; and

$HACON_x$ = "Herbicides Applied on Conventional" hectares (kgs a.i./crop year).

The first four of the above-five variables are reported or can be derived from USDA data; the fifth can be calculated by solving the above equation for $HACON_x$. For each HR crop and year combination, the impact of HR cultivars on average herbicide use is calculated by subtracting $HAHT_x$ from $HACON_x$. This difference is then multiplied by the HR hectares planted, to calculate the impact of HR crops on herbicide use in a given year. Increases or decreases in the volume of herbicides applied as a result of the planting of HR crops are then aggregated across all years (1996–2011) and the three HR crops.

In the case of *Bt* transgenic corn, the average rate of application of insecticides targeting the ECB and the CRW must be calculated. This process is complicated by the fact that several insecticides are applied for control of the ECB and CRW, as well as other insects. Pesticide labels, treatment recommendations in university spray guides, and experts in corn Integrated Pest Management (IPM) were consulted in carrying out this step [Additional file 1: Tables S11, S12].

Average rates of insecticide application across all corn hectares treated per crop year are then calculated, weighted by portions of total hectare treatments. This weighted-average rate of insecticide application on hectares treated for ECB control declines from 0.24 kgs/ha (0.21 pounds/acre) of active ingredient in 1996 to 0.15 kgs/ha (0.13 pounds/acre) in 2010. In the case of CRW insecticides, the rate falls from 0.76 kgs/ha in 1996 to 0.2 kgs/ha in 2010.

The next step in calculating the pounds of insecticides displaced by the planting of *Bt* corn is to estimate the portion of hectares planted to *Bt* corn for ECB and/or CRW control that would have been treated with an insecticide if the corresponding *Bt* crop had not been planted. Doing so requires a set of assumptions and projections.

Historically, USDA data shows that before the advent of *Bt* corn, 10% +/− 3% of national corn hectares were treated for ECB control, while 27% +/− 4% were treated for CRW control. Yet by 1998 (third year of commercial sales), 19% of corn hectares were planted to a *Bt* cultivar targeting the ECB – about double the historic share of hectares treated with an insecticide for this pest. Today, close to two-thirds of corn hectares are planted to *Bt* for ECB cultivars, some six-times the historic rate. In the case of *Bt* corn for CRW, by the fifth year of commercial sales, 2007, the share of corn hectares planted to CRW hybrids was 25.6%, roughly equaling the historic share of hectares treated with CRW insecticides (27% +/− 4%). In 2011, 60% of corn hectares were planted to a CRW hybrid, double the historic share of corn hectares treated with a CRW insecticide.

The impact of *Bt* corn on the volume of insecticide displaced per hectare should be adjusted downward to account for hectares that would, in all likelihood, not have been treated. In the case of *Bt* corn targeting the ECB, the likely share of hectares planted to *Bt* corn that would have been sprayed for ECB control begins at 90% in 1997, the first year of commercial planting, and drops incrementally to 45% in 2007.

This percent is left unchanged from 2008–2010, despite the increase in corn hectares planted to *Bt* corn for ECB from 49% to 65%, because of reported increases in insect pest pressure in major corn producing regions [49]. The result is the projection that in 2011, insecticide applications were displaced on 10.9 million hectares of corn (27 million acres) planted to *Bt* hybrids for ECB control (45% of the 65% of corn hectares planted to *Bt* for ECB hybrids). These 10.9-million hectares are 29% of total corn hectares planted, and is about three-times the historic level of insecticide applications for ECB control.

In the case of *Bt* corn for CRW control, the percent of hectares planted that displaces insecticide use begins at 95% in 2003, the first year of commercial sales, and

declines to 55% in 2011. In 2011, 57% of corn hectares were planted to a *Bt* CRW hybrid, and hence *Bt* corn for CRW displaced insecticide use on 31% of national hectares planted. This estimate assumes that any hectare planted to a *Bt* corn for CRW control was not also treated with a CRW insecticide. In addition, 9.4% of corn hectares were sprayed for CRW control with an insecticide. Accordingly, about 40% of corn hectares were either sprayed for the CRW or planted to a *Bt* variety for CRW control, well above the 27% +/− 4% level treated with insecticide over the last 20 years.

The historically high, projected level of CRW treatment is justified, in part, by the emergence in the late 1990s of a variant of the CRW that learned to overwinter in soybean fields, thus undermining the efficacy of corn-soybean rotations in reducing CRW populations [50]. Recent, historically high corn prices have also increased the frequency of continuous corn, a management factor that surely has increased CRW pressure.

Bt cotton targets the budworm/bollworm complex, but does not affect other insect pests, including the boll weevil, plant bugs, white flies, and stinkbugs. Applications of broad-spectrum insecticides are typically made on essentially 100% of planted cotton hectares to control the budworm/bollworm complex and other insects. *Bt* cotton will reduce the use of insecticides on the budworm/bollworm complex, but will only indirectly impact applications of insecticides targeting other insects.

[Additional file 1: ST 13] reports the basis for estimating the pounds of insecticides displaced by each acre planted to *Bt* cotton. University insect management guides and experts were consulted to estimate the portion of hectares treated with each cotton insecticide that targeted the budworm/bollworm complex, versus other insects. The number of acres treated with each insecticide is calculated from NASS data, as well as the share of total acres treated. Average insecticide use rates are then calculated, weighted by each active ingredient's share of insecticide acre treatments targeting the budworm/bollworm complex. The weighted average cotton insecticide application rate falls modestly from 0.46 kgs/ha (0.41 pounds/acre) in 1997 to 0.27 kgs/ha (0.24 pounds/acre) in 2010–2011.

Table 4 summarizes the basis for projections required to estimate the volume of insecticide use displaced by the planting of a hectare to *Bt* corn or cotton cultivars.

Additional file

Additional file 1: The projection model used is composed of a series of linked worksheets in a Microsoft Excel workbook. Each table within the workbook appears below in pdf as sequentially numbered Additional file 1: Table S1 (e.g., ST 1). The pesticide use data incorporated in the model were originally reported by U.S. government agencies in pounds of active ingredient, and/or pounds of a.i./acre, and so these units are used throughout the Additional files to report data on herbicide use. Convert pounds to kgs by multiplying by 0.454; to convert pounds/acre to kg/ha, multiply by 1.12.

Abbreviations

AI: Active ingredient; *Bt*: Bacillus thuringiensis; CRW: Corn rootworm; ECB: European corn borer; EPSPS: Enolpyruvylshikimate-3-phosphate synthase; EPA: Environmental Protection Agency; ERS: Economic Research Service; GE: Genetically engineered, genetic engineering; GR: Glyphosate resistant; ha: Hectare; HR: Herbicide Tolerant; IPM: Integrated Pest Management; kgs: Kilograms; NASS: National Agricultural Statistics Service; NCFAP: National Center for Food and Agriculture Policy; RR: Roundup Ready; SI: International System of Units; ST: Supplemental Table; THA: Total hectares; US: United States; USDA: United States Department of Agriculture; WSSA: Weed Science Society of America.

Competing interests

The author declares he has no competing interests.

Author contribution

Charles Benbrook (CB) developed the model, carried out the analysis, and wrote the paper. The author read and approved the final manuscript.

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Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of *Bt* maize[1]

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- *Premise of the study:* Insect-resistant *Bacillus thuringiensis* (*Bt*) maize is widely cultivated, yet few studies have examined the interaction of symbiotic arbuscular mycorrhizal fungi (AMF) with different lines of *Bt* maize. As obligate symbionts, AMF may be sensitive to genetic changes within a plant host. Previous evaluations of the impact of *Bt* crops on AMF have been inconsistent, and because most studies were conducted under disparate experimental conditions, the results are difficult to compare.
- *Methods:* We evaluate AMF colonization in nine *Bt* maize lines, differing in number and type of engineered trait, and five corresponding near-isogenic parental (P) base hybrids in greenhouse microcosms. Plants were grown in 50% local agricultural soil with low levels of fertilization, and AMF colonization was evaluated at 60 and 100 d. Nontarget effects of *Bt* cultivation on AMF colonization were tested in a subsequently planted crop, *Glycine max*, which was seeded into soil that had been preconditioned for 60 d with *Bt* or P maize.
- *Key results:* We found that *Bt* maize had lower levels of AMF colonization in their roots than did the non-*Bt* parental lines. However, reductions in AMF colonization were not related to the expression of a particular *Bt* protein. There was no difference in AMF colonization in *G. max* grown in the *Bt*- or P-preconditioned soil.
- *Conclusions:* These findings are the first demonstration of a reduction in AMF colonization in multiple *Bt* maize lines grown under the same experimental conditions and contribute to the growing body of knowledge examining the unanticipated effects of *Bt* crop cultivation on nontarget soil organisms.

Key words: arbuscular mycorrhizal fungi; *Bacillus thuringiensis*; Cry1Ab; Cry34/35Ab1; Cry3Bb1; Cry1F; *Glycine max*; soybean; transgenic; *Zea mays*.

Genetically modified (GM) crops, engineered to express herbicide-tolerance, insecticidal properties, or a combination of traits, are the most rapidly adopted agricultural biotechnology in recent history (James, 2010). Since their commercial introduction in 1996, the global adoption of GM crop technology has increased ca. 87-fold, up from 1.7 million hectares in 1996 to 148 million hectares in 2010 (James, 2010). Insect-resistant maize (*Zea mays* L.), one of the most widely cultivated GM crops, is engineered to express insecticidal toxins derived from the spore-forming soil bacterium *Bacillus thuringiensis* (*Bt*). To date, more than 60 different *Bt* crystal proteins (called Cry proteins) that exhibit a high degree of specificity toward certain insect pests have been identified (reviewed in Schnepf et al., 1998; Federici, 2002; Stotzky, 2002; Lee et al., 2003; Icoz and Stotzky, 2008b; Sanchis, 2011). *Bt* crops that provide resistance to multiple agricultural pests, as well as confer herbicide-tolerance, have contributed to the popularity of GM crops among farmers worldwide (EPA, 2011). In 2010, 86% of the maize grown in the USA (USDA, 2010) and 26% of the global biotech hectarage was cultivated in maize genetically modified to express one or more engineered traits (James, 2010). This rapid and widespread adoption of GM crops has led to a dramatic shift in the agricultural landscape over the last 15 years and has raised questions about the impact of insect-resistant *Bt* crops on nontarget organisms in the soil environment.

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that have been shown to improve plant nutrient acquisition, especially in low nutrient soil environments (e.g., Galvez et al., 2001; Gosling et al., 2006; Lekberg et al., 2008; Sheng et al., 2008). These symbiotic fungi are ubiquitous in soil and are found in both natural and agroecosystems (Smith and Read, 2008). Because AMF rely on a plant host for nutrition and reproduction, they may be sensitive to changes in the physiology of the host plant, to biochemical changes associated with the *Bt* modification, or to alterations in root exudates released into the rhizosphere. Although *Bt* proteins are expressed in the roots of most *Bt* maize lines (Saxena and Stotzky, 2000; Saxena et al., 2002; reviewed by Icoz and Stotzky, 2008a, b; EPA, 2011), the evidence that Cry proteins have a direct effect on AMF is equivocal. For example, lower AMF colonization levels have been reported in *Bt* maize lines *Bt* 11 (Castaldini et al., 2005; Cheeke et al., 2011) and *Bt* 176 (Turrini et al., 2004; Castaldini

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et al., 2005) expressing Cry1Ab, but *Bt* maize (MON810) expressing the same Cry1Ab protein did not have lower AMF colonization when compared to its non-*Bt* parental isoline (de Vaufleury et al., 2007). There were also no negative effects on AMF reported for *Bt* cotton expressing Cry1Ac and Cry2Ab (Knox et al., 2008). However, AMF colonization was significantly lower in *Medicago sativa* grown for 4 months in soil amended with *Bt* 11 maize compared with *M. sativa* grown in soil amended with non-*Bt* maize (Castaldini et al., 2005). Because these studies were conducted under different experimental conditions with variations in AMF inocula, *Bt* cultivar, Cry protein, fertilizer level, harvest time, and assessment method, it has been difficult to compare results across studies. Moreover, the reduction in AMF colonization observed in certain *Bt* maize lines may also be due to indirect effects of the gene insertion, which may cause a change in root exudates or biochemical composition of the plant tissue, rather than to a direct effect of Cry protein on soil fungi (e.g., Naef et al., 2006; Devare et al., 2007). Given the initial indication that some lines of *Bt* maize are poorly colonized by AMF (Turrini et al., 2004; Castaldini et al., 2005; Cheeke et al., 2011) and that results to date have been inconsistent across studies, it is important to determine whether *Bt* maize lines expressing different numbers and types of engineered traits have a negative effect on arbuscular mycorrhizal fungi when evaluated under the same experimental conditions.

In this greenhouse study, we addressed three specific questions: (1) Will a difference in AMF colonization be detected between different *Bt* and non-*Bt* maize lines grown under the same experimental conditions? (2) If so, are these differences related to the expression of a particular *Bt* protein? (3) Does *Bt* maize cultivation have a negative effect on AMF colonization of a subsequently planted crop? To address the first two questions, we examined AMF colonization in nine *Bt* maize lines, differing in number and type of engineered trait, and five corresponding non-*Bt* near isogenic parental (P) base hybrids (Table 1) at two different time points in the maize lifecycle. To investigate whether *Bt* crop cultivation has a negative impact on AMF colonization of a subsequently planted species, *Glycine max* (L.) Merr (vegetable soybean; Sayamusume) was grown to maturity in soil that had been preconditioned for 60 d with *Bt* or non-*Bt* maize. We hypothesized that AMF colonization would be lower in the *Bt* maize lines (Turrini et al., 2004; Castaldini et al., 2005; Cheeke et al., 2011) and that AMF colonization would also be reduced in *G. max* grown in soil preconditioned with *Bt* maize (Castaldini et al., 2005). The consistent experimental conditions used in this study were optimized to reflect low-input agricultural systems to allow for maximal AMF colonization

TABLE 1. Fourteen different *Bt* and non-*Bt* maize lines, representing a cross section of the broad range of *Bt* maize lines commercially available, were evaluated for AMF colonization in greenhouse microcosm experiments. Before planting, the *Bt* maize hybrids were assigned numbers B1–B9, and their corresponding non-*Bt* parental base hybrids were assigned numbers P1–P5. Note that P2 was the parental line for B2 and B5, P3 was the parental line for B3 and B6, and P5 was the parental line for B7, B8, and B9. The *Bt* maize cultivars that express the same proteins differ in the background genetics of their parental line.

Bt no.	Company; Plant ID	Cry protein	Protection	Maize type	Parental isoline (P) no.
B1	Syngenta; Attribute, *Bt* 11: BC0805	Cry1Ab	European corn borer protection, corn ear worm, fall armyworm	Triple sweet hybrid sweet corn	P1 [a]
B2	N/A [b]	Cry34/35Ab1	Western corn rootworm, northern corn rootworm, and Mexican corn rootworm protection; glufosinate tolerance; glyphosate tolerance	Field corn	P2
B3	N/A [b]	Cry34/35Ab1	Western corn rootworm, northern corn rootworm, and Mexican corn rootworm protection; glufosinate tolerance	Field corn	P3
B4	N/A [b]	Cry1F Cry34/35Ab1	Western bean cutworm, corn borer, black cutworm and fall army worm resistance; glufosinate tolerance. Western corn rootworm, Northern corn rootworm protection; glyphosate tolerance	Field corn	P4
B5	N/A [b]	Cry1F	Western bean cutworm, corn borer, black cutworm and fall armyworm resistance; glyphosate tolerance; glufosinate tolerance	Field corn	P2
B6	N/A [b]	Cry1F	Western bean cutworm, corn borer, black cutworm and fall armyworm resistance; glyphosate tolerance; glufosinate tolerance	Field corn	P3
B7	Monsanto; DKC51-41 Mon 863, Nk603 [c]	Cry3Bb1	Corn rootworm protection; glyphosate tolerance (RR2)	Field corn	P5, DKC51-45 (RR2)
B8	Monsanto; DKC50-20 Mon 810, Nk603 [c]	Cry1Ab	European corn borer protection; glyphosate tolerance (RR2)	Field corn	P5, DKC51-45 (RR2)
B9	Monsanto; DKC51-39 Mon 863, Mon 810, Nk603 [c]	Cry1Ab Cry3Bb1	Corn rootworm, European corn borer protection; glyphosate tolerance (RR2)	Field corn	P5, DKC51-45 (RR2)

[a] The *Bt* 11 transgene was backcrossed into one of the parents of Providence (P1) to create the variety BC0805. This *Bt* 11 cultivar was transformed using plasmid pZ01502 (containing Cry1Ab, pat, and amp genes) to express the Cry1Ab protein of *Bt*.

[b] Our seed agreement prohibits us from disclosing information about this seed industry representative, the genetics of the *Bt* and parental isolines, or other information related to the seeds provided for this study.

[c] Nk603 is the gene for Round Up Ready 2 (RR2) glyphosate tolerance.

[d] Information on plant ID, cry protein, protection, and maize type was obtained from the seed suppliers and the U.S. Environmental Protection Agency Current & Previously Registered Section 3 PIP Registrations.

(e.g., Cheeke et al., 2011), and locally collected agricultural soil was used to evaluate how each *Bt* and non-*Bt* maize cultivar responds to a natural community of AMF in the soil.

MATERIALS AND METHODS

***Experimental overview*—**In the first phase of this study, microcosms were constructed with a common soil community (50% local agricultural soil, 25% sterile sand, and 25% sterile soil-less potting media) and cultured with one *Bt* or non-*Bt* maize host plant, with 10 replicates of each cultivar (one plant in 10 separate 4-L pots), for a total of 140 plants in the experiment. After establishing a vegetative history in each microcosm for 60 d, five replicates of each *Bt* and P maize line were destructively harvested, and roots were assessed for AMF colonization (McGonigle et al., 1990). *Glycine max* was then seeded into each preconditioned microcosm and destructively harvested at maturity to determine whether AMF colonization would be reduced in plants grown in soil preconditioned with *Bt* maize. The five remaining replicates of each maize line were harvested at day 100 to assess AMF colonization at a different physiological time point in the maize lifecycle (when plants had started to produce ears). Growth responses (height, leaf number, chlorophyll content, root biomass, shoot biomass, and ear number) were recorded to determine whether plants with higher levels of AMF colonization exhibited any growth or yield benefits as a result of the symbiosis.

***Plant cultivars*—**Nine different lines of *Bt* maize (*Zea mays*) and five corresponding non-*Bt* parental base hybrids were obtained from three seed companies (Syngenta Seeds, Boise, Idaho, USA; Monsanto, St. Louis, Missouri, USA, and an additional representative seed industry seed supplier). Before planting, the *Bt* maize lines were assigned numbers B1–B9, and their corresponding non-*Bt* parental base hybrids were assigned numbers P1–P5. Note that some non-*Bt* isolines were the base-genetics for more than one *Bt* line; P1 was the base hybrid for B1, P2 was the base hybrid for B2 and B5, P3 was the base hybrid for the B3 and B6, P4 was the base hybrid for B4, and P5 was the base hybrid for B7, B8, and B9. The *Bt* maize lines obtained for this study differed in type (sweet corn or field corn), the *Bt* protein expressed (Cry1Ab, Cry34/35Ab1, Cry1F + Cry34/35Ab1, Cry1F, Cry3Bb1, Cry1Ab + Cry3Bb1), the number and type of inserted traits (insect protection: European corn borer, corn root worm, Mexican corn worm, western bean cutworm, black cutworm, fall armyworm, among others; herbicide protection: glufosinate and/or glyphosate tolerance), and background genetics, representing a cross-section of the broad range of *Bt* maize lines commercially available (Table 1). The non-*Bt* parental maize seeds obtained from Monsanto are the corresponding parental lines to the *Bt* lines and were described as non-*Bt* near isoline control hybrids; and the corresponding non-*Bt* maize seeds obtained from Syngenta and the other seed industry supplier were described as near-isogenic parental base hybrids or parental isolines. We are prohibited by our seed agreement from disclosing more information about the background genetics, gene expression, *Bt* protein concentration, parental isolines, or other details related to genetics of these plant lines (both genetically modified and parental). For simplicity, we will refer to all *Bt* maize plants in this study as (*Bt*) and the non-*Bt* maize plants as parentals (P). The nongenetically modified *G. max* seeds used in the second phase of the experiment were obtained from Territorial Seed Co. (Cottage Grove, Oregon, USA) and were chosen to represent the corn-soybean rotation commonly practiced in the USA.

***Test of soil nutrients and AMF spore composition*—**Soil was collected from a certified organic field plot (previously sown in mixed vegetables) in March 2008 at the Washington State University Research and Extension Center (Vancouver, Washington, USA) and analyzed for nutrients (24 ppm nitrogen ($N0_3$-N), 108 ppm phosphorus (Weak Bray), 474 ppm potassium), percentage organic matter (4.5%), soil texture (silt loam), and soil pH (6.1) by an independent laboratory (A&L Western Agricultural Laboratories, Portland, Oregon, USA). Prior to planting, spores were extracted from a composite sample of the agricultural soil and identified morphologically at the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (Morgantown, West Virginia, USA). In the agricultural soil, spores were identified that represented six putative AMF taxa: *Gigaspora rosea* or *Gi. albida*, *Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum*, *Paraglomus occultum*, and an undescribed *Acaulospora*.

For this study, we chose to use endogenous AMF inoculum from whole soil rather than defined additions of AMF spores or single species cultures. Inoculations with single AMF species or a specific number of spores provide limited information about how a plant might respond to a community of AMF in a natural or agroecosystem and give little insight into the plant–fungal associations that are likely to be encountered in the field. The use of endogenous mycorrhizal inocula in whole soil is more ecologically relevant than using defined additions of AMF spores or single species AMF cultures and is more useful for predicting how different lines of *Bt* maize might respond to a natural community of AMF under field conditions. For effects of single species cultures on AMF colonization in *Bt* maize, see Cheeke et al. (2011), Castaldini et al. (2005), and Turrini et al. (2004).

***Construction of microcosms*—**This experiment commenced in March 2008 in a research greenhouse at Portland State University (Portland, Oregon, USA). Seeds of each *Bt* and P maize cultivar were surface sterilized in a 10% bleach solution and planted into 4-L nursery pots containing a hand-mixed potting mix of 50% nonsterile agricultural soil (Vancouver, Washington, USA), 25% sterile sand, 25% sterile Sunshine Mix soil-less potting medium (70–80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum, wetting agent; Sun Gro Horticulture, Bellevue, Washington, USA), with the agricultural soil serving as the natural AMF inoculum. Ten replicates of each plant line were planted (one plant in 10 separate 4-L pots, representing 14 *Bt* and P lines), for a total of 140 maize plants in the experiment.

***Growth conditions and fertilizer treatments*—**To account for microclimatic effects, we set up pots in a completely randomized design and rotated on the greenhouse bench each week using a randomization key. The daytime temperatures in the greenhouse were between 27°C and 32°C and nighttime temperatures were between 20°C and 27°C, which reflect growing temperatures of many corn-growing regions in the USA. Photoperiod lasted from 0600 to 2000 hours every day, supplied via metal halide lights and natural sunlight. Humidity varied between 50 and 70% throughout the growing period. Plants were hand watered daily and fertilized every 2 wk with 200 mL of a dilute fertilizer (0.23 g/L of Peter's Professional All Purpose Plant Food 24-8-16, St. Louis, Missouri, USA).

***Assessment of maize plant growth*—**Maize plant height and leaf number were recorded 2 wk after planting, and at day 30, 60, and 100. After root samples had been collected for AMF assessment, shoots and roots were separated and dried for at least 48 h at 60°C for biomass data. Chlorophyll (Chl) content was collected from live leaves (Minolta SPAD-502 Leaf Chl meter, Osaka, Japan), and the number of ears on each maize plant was recorded at day 100.

***Test of Bt-preconditioned soil on AMF colonization in G. max*—**After harvesting the 60 d maize plants, the soil microcosms were stored on a greenhouse bench for 30 d, mimicking the rest period between when one *Bt* crop is harvested and a different crop is planted. *Glycine max* was grown to maturity in five replicate pots containing soil that had been pre-exposed for 60 d with one *Bt* or non-*Bt* maize line. At harvest, data were collected on *G. max* height, root and shoot biomass (dry mass), bean pod number, and percent AMF colonization of roots.

***Mycorrhizal fungus colonization assessment*—**At harvest, roots were rinsed in tap water to remove soil particles and an equivalent amount of cut samples were taken from each root system. Roots were cleared using 10% KOH, neutralized in 2% aqueous HCl, and stained with 0.05% w/v trypan blue in lactoglycerol to visualize fungal structures (Phillips and Hayman, 1970), and at least 50 cm of roots from each plant were scored for mycorrhizal fungus colonization using the slide-intersect method (McGonigle et al., 1990). So that the researcher was not aware of which root type (*Bt* or non-*Bt*) was being analyzed at the time of data collection, histocassettes were mixed randomly, and slides were labeled when they were being prepared using a sequential number system that was not in any way associated with the *Bt* or P treatment. The presence or absence of hyphae, arbuscules, and vesicles observed per 100 root intersects was recorded for each sample. Total percentage AMF colonization was recorded as the total number of intersects out of 100 that had the presence/absence of any fungal structure (hyphae, arbuscules, and/or vesicles).

***Data analysis*—**Differences in arbuscular mycorrhizal fungal colonization (hyphae, arbuscules, vesicles, and total percentage AMF colonization) and plant growth responses between *Bt* and P maize ($\alpha = 0.05$) were analyzed using the Proc Mixed procedure of SAS version 9.1 (SAS Institute, Cary, North Carolina, USA). The Proc GLM procedure of SAS version 9.1 was also performed for each analysis, but because the significant results were similar, we only included

the Proc Mixed results here. To test for differences in AMF colonization between *Bt* and P maize, *Bt* was treated as a fixed effect and parental and *Bt* × parental were treated as random effects. To test for differences in plant growth responses at 60 d (root biomass and shoot biomass) and 100 d (root biomass, shoot biomass, chlorophyll content of fresh leaves, and ear number per plant), *Bt*, initial plant size (plant height × leaf no.), and AMF colonization levels were treated as fixed effects, and parental and *Bt* × parental were treated as random effects. To test for differences in AMF colonization as affected by specific Cry protein, the influence of the parental lines were controlled for in the model by entering the average level of AMF colonization in the parental as a covariate, and each Cry protein was treated as a fixed effect for both the 60 and 100 d harvest. AMF data were arcsine square-root transformed for each analysis, and maize root biomass was square-root transformed for the 60 d analysis to meet the assumptions of the model.

The Proc Mixed procedure of SAS was used to test for differences in AMF colonization in *G. max* grown in soil preconditioned with *Bt* or non-*Bt* maize. For the test of soil feedback on AMF colonization in *G. max*, the fixed effect was soil (soil pre-exposed for 60 d with a *Bt* or P maize cultivar). For the analysis of *G. max* growth responses (root biomass, shoot biomass, and bean pod number) in the preconditioned soil, the fixed effects were soil and AMF.

RESULTS

***Effect of maize cultivar on AMF colonization*—**At the 60-d harvest when plants were actively growing, AMF colonization of roots was significantly lower in the *Bt* maize lines compared with the non-*Bt* parental maize plants ($F_{1,4}$ = 9.0, P = 0.04; Fig. 1). When analyzed by fungal structure, colonization by hyphae ($F_{1,4}$ = 5.63, P = 0.08), arbuscules ($F_{1,4}$ = 6.46, P = 0.06), and vesicles ($F_{1,4}$ = 1.03, P = 0.37) did not differ statistically between the *Bt* and non-*Bt* maize lines (Fig. 1). At the 100-d harvest when plants were starting to produce ears, percentage colonization by arbuscules was significantly lower in the *Bt* maize lines ($F_{1,4}$ = 9.25, P = 0.04) compared to the non-*Bt* parental lines (Fig. 2). There was no significant difference in hyphal colonization ($F_{1,4}$ = 1.42, P = 0.30), vesicles ($F_{1,4}$ = 0.02, P = 0.89), or total percentage AMF colonization ($F_{1,4}$ = 3.39, P = 0.14) detected between the *Bt* and non-*Bt* maize lines at the second harvest period when plants were near maturity (Fig. 2). Across all maize lines, percentage AMF colonization was lower at the 100-d harvest when plants were producing ears than when they were in an active growth phase at the 60-d harvest (Figs. 1, 2).

***Effect of AMF colonization and cultivar type on maize growth*—**At 60 d, percentage AMF colonization was negatively correlated with shoot biomass (Pearson correlation coefficient = −0.37, P = 0.002; Proc mixed $F_{1,58}$ = 4.68, P = 0.03), but there was no effect of AMF colonization on root biomass ($F_{1,57}$ = 0.23, P = 0.63). There was no difference in root biomass ($F_{1,4}$ = 0.72, P = 0.44) or shoot biomass ($F_{1,4}$ = 0.27, P = 0.63) between the *Bt* and non-*Bt* maize cultivars at the 60-d harvest.

At the 100-d harvest, there was no effect of AMF colonization on root biomass ($F_{1,58}$ = 1.53, P = 0.22), shoot biomass ($F_{1,58}$ = 3.83, P = 0.06), or chlorophyll content of fresh leaves ($F_{1,58}$ = 0.13, P = 0.72). However, maize plants with higher levels of AMF colonization had fewer ears ($F_{1,58}$ = 3.88, P = 0.05) at the 100-d harvest than plants with lower levels of AMF colonization. There was no difference in shoot biomass ($F_{1,4}$ = 0.03, P = 0.87), ear number ($F_{1,4}$ = 0.11, P = 0.75), or chlorophyll content of fresh leaves ($F_{1,4}$ = 0.02, P = 0.89) between the *Bt* and non-*Bt* maize cultivars, although the *Bt* maize plants had a significantly greater root biomass ($F_{1,4}$ = 9.19, P = 0.04) than the non-*Bt* parental plants at the 100-d harvest. Initial plant size

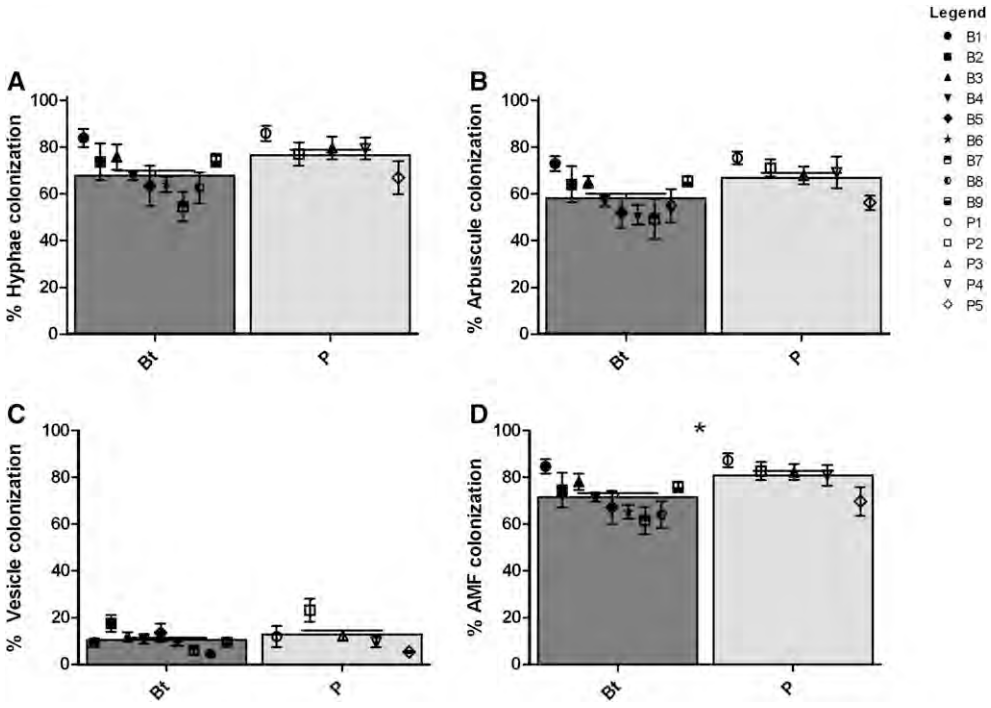

Fig. 1. Mean percentage incidence (±SE) of (A) AMF hyphal colonization, (B) arbuscule colonization, (C) vesicle colonization, and total percentage of (D) AMF colonization (per 100 intersects on root sample) in *Bt* and non-*Bt* parental (P) maize plants grown for 60 d in a greenhouse in 50% locally collected agricultural soil. Dark gray bars represent means (±SE) of pooled *Bt* AMF data (N = 45); light gray bars represent means (±SE) of pooled P AMF data (N = 25); *$P \le 0.05$. Symbols represent means (±SE) of individual *Bt* and P maize lines; N = 5 for each symbol. P1: base-parental for B1, P2: parental for B2 and B5, P3: parental for B3 and B6, P4: parental for B4, P5: parental for B7, B8, and B9.

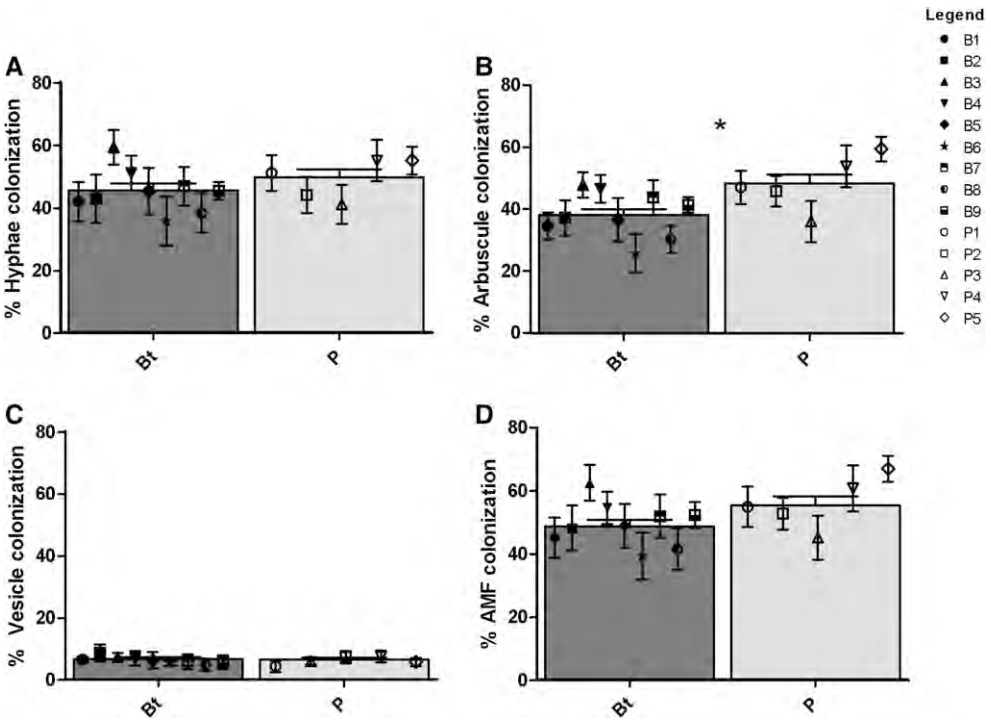

Fig. 2. Mean percentage incidence (±SE) of (A) AMF hyphal colonization, (B) arbuscule colonization, (C) vesicle colonization, and total percentage (D) AMF colonization (per 100 intersects on root sample) in *Bt* and non-*Bt* parental (P) maize plants grown for 100 d in a greenhouse in 50% locally collected agricultural soil. Dark gray bars represent means (±SE) of pooled *Bt* AMF data ($N = 45$); light gray bars represent means (±SE) of pooled P AMF data ($N = 25$); $*P \leq 0.05$. Symbols represent means (±SE) of the individual *Bt* and P maize lines; $N = 5$ for each symbol. P1: base-parental for B1, P2: parental for B2 and B5, P3: parental for B3 and B6, P4: parental for B4, and P5: parental for B7, B8, and B9.

(height × leaf number) was the best predictor of root biomass ($F_{1,57} = 18.57$, $P < 0.0001$; $F_{1,58} = 18.10$, $P < 0.0001$) and shoot biomass ($F_{1,58} = 50.42$, $P < 0.0001$; $F_{1,58} = 10.62$, $P = 0.002$) at 60 and 100 d, respectively, for both *Bt* and P plants.

***Effect of type of Cry protein expressed on AMF colonization in* Bt *maize*—**The type of Cry protein expressed in the different *Bt* maize lines was generally not a strong predictor of AMF infection among the *Bt* cultivars (Table 2). When controlled for the influence of the parental lines in the analysis, *Bt* maize lines expressing Cry1Ab had higher AMF infection levels (hyphae, arbuscules, and total AMF) than other *Bt* lines at the 60-d harvest, but this was primarily driven by the high AMF colonization in the B9 cultivar (Fig. 1A, B, D). *Bt* maize lines expressing Cry1F had lower arbuscule colonization compared to the other *Bt* maize lines at 60 d (Table 2; Fig. 1B). At the 100-d harvest, *Bt* maize lines expressing Cry34/35Ab1 had higher AMF colonization levels (hyphae, arbuscules, vesicles, and total AMF) in roots compared with the other *Bt* maize lines (Table 2; Fig. 2). The best predictor of AMF infection in the different *Bt* lines at the 60-d harvest was the AMF infection level of the associated parental lines ($F_{1,34} = 11.30$; $P = 0.002$). There was no effect of parental line on AMF colonization detected at the 100 d harvest ($F_{1,34} = 0.00$; $P = 0.99$). Regardless of the specific type of Cry protein(s) expressed, *Bt* maize lines overall had lower AMF colonization than their non-*Bt* parental lines at the 60-d harvest (Fig. 1) and lower colonization by arbuscules at the 100-d harvest (Fig. 2).

***Effect of soil preconditioned with* Bt *or P maize on AMF colonization, plant growth, and yield in vegetable soybean*—**When *G. max* was grown to maturity in soil preconditioned for 60 d with a *Bt* or non-*Bt* maize plant, there was no effect of the *Bt*-preconditioned soil on arbuscular mycorrhizal colonization of

TABLE 2. Proc Mixed results (*F*-values) of effects of Cry protein on percentage incidence of hyphae, arbuscules (Arb), vesicles, and total AMF colonization on root samples at the 60- and 100-d harvest. The influence of the parental lines was controlled for in the model by entering the mean level of AMF colonization in the parental as a covariate.

		60-d harvest				100-d harvest			
Cry protein	df	Hyphae	Arb	Vesicles	Total AMF	Hyphae	Arb	Vesicles	Total AMF
Cry1Ab	1,34	5.47*	7.02**	0.22	4.57*	1.39	1.61	0.74	1.35
Cry34/35Ab1	1,34	0.84	1.41	0.89	1.03	5.55*	6.31*	4.00*	5.39*
Cry3Bb1	1,34	0.65	0.25	0.42	0.00	0.23	2.66	0.15	0.80
Cry1F	1,34	1.64	4.11*	0.08	2.52	0.29	0.99	0.14	0.55

Notes: $*P \leq 0.05$, $**P \leq 0.01$

G. max roots ($F_{1,4}$ = 0.18, P = 0.69), nor was there an effect of the preconditioned soil on *G. max* root biomass ($F_{1,4}$ = 0.33, P = 0.59), shoot biomass ($F_{1,4}$ = 0.40, P = 0.56), or bean pod number at harvest ($F_{1,4}$ = 0.47, P = 0.53).

DISCUSSION

Genetically modified *Bt* maize and the non-*Bt* parental lines differed in their level of mycorrhizal colonization in roots when grown in field-collected soil containing a natural community of AMF. When maize plants were in a period of active growth, total AMF colonization was significantly lower in the *Bt* maize lines compared to the non-*Bt* parental lines. When the maize plants were closer to maturity and starting to produce ears, arbuscule formation was lower in the *Bt* maize cultivars. Although there was some variation in mycorrhizal infection levels within the different *Bt* maize and non-*Bt* parental lines, the *Bt* maize cultivars collectively exhibited lower AMF colonization compared to the parental lines, regardless of the number or type of engineered trait, their genetic background, or the type of Cry protein(s) expressed. Moreover, as there was no difference in AMF colonization of *G. max* grown in the *Bt* or non-*Bt* maize preconditioned soil, this study supports other research indicating that reductions in AMF colonization are likely not a result of a direct toxic effect of *Bt* proteins (Donegan et al., 1995; Koskella and Stotzky, 2002; Ferreira et al., 2003), but may be a result of other factors, such as an indirect effect of the genetic insertion within each *Bt* plant line (e.g., Donegan et al., 1995; Flores et al., 2005; Naef et al., 2006) that may affect their ability to respond to or recruit AMF in the rhizosphere, or as a result of differences in the background germplasm of the parental line that may influence how derived lines interact with AMF and/or acquire nutrients in the soil.

Variations in AMF colonization levels have been reported in other crop varieties (e.g., maize, wheat) (Hetrick et al., 1992; Kaeppler et al., 2000; Sawers et al., 2008), including commercial maize lines that were selected under conditions of high phosphorus fertilization (Kaeppler et al., 2000), but it is not clear why the *Bt* maize lines in this study had lower levels of AMF in their roots than the non-*Bt* controls at two different harvest periods. The genetic basis of mycorrhizal responsiveness has been documented in a variety of agricultural crop species including rice (Gao et al., 2007), wheat (Hetrick et al., 1992), and maize (Kaeppler et al., 2000), as well as in wild species such as big bluestem (Schultz et al., 2001) and St. John's wort (Seifertet al., 2009), so it is possible that the insertion of the *Bt* construct in different *Bt* maize lines could affect the plant–fungal symbiosis in some GM cultivars, although this is difficult to determine with the design of the current study. Pleiotropic effects (change in a single gene that affects multiple phenotypic traits) of a genetic insertion are not uncommon (e.g., Sheveleva et al., 1998; reviewed in Wang et al., 2003) and certain types of genetic changes, such as those that influence physiology (e.g., sugar allocation, enzyme activity in roots, lignin content) may affect the ability of some *Bt* maize lines to form relationships with AMF. Alternatively, AMF colonization levels in the *Bt* maize roots may also be strongly influenced by the background genetics of the parental line. At the 60-d harvest, for example, the best predictor of AMF infection in the *Bt* lines was the infection level of the associated parental line. However, this does not explain why AMF colonization was lower in the *Bt* cultivars compared with the non-*Bt* parental maize lines when grown under the same conditions. Given that there is likely still a certain amount of variation between each *Bt* line and its near isogenic parental base hybrid, more work should be conducted to explore possible mechanisms that may contribute to the lower levels of AMF colonization observed in multiple *Bt* maize lines.

We did not observe growth benefits for maize plants that had higher levels of AMF colonization in their roots at either 60 or 100 d. In fact, maize plants that had higher AMF colonization had reduced shoot biomass at 60 d and a lower ear number at 100 d. A negative effect of AMF on maize biomass has also been observed in other studies; maize plants grown in high phosphorus treatments with AMF had 88% of the above ground biomass of maize plants grown at high phosphorus treatments without AMF, indicating that the AMF symbiosis can reduce plant biomass under certain growth conditions (Kaeppler et al., 2000). It is well known that the plant-AMF symbiosis is dynamic and can range from parasitism to mutualism depending on the growth stage of the plant, ecological conditions, differences in cultivation practices, and many other biotic and abiotic factors (Johnson et al., 1997; Kiers et al., 2002; Hirsch, 2004; Jones and Smith, 2004). Because we grew these plants in a fixed-volume of soil under low-fertilizer conditions in the greenhouse, it is not known how the *Bt* and non-*Bt* maize lines in our study would respond to AMF in the field. However, it has been shown that even when no plant growth responses are detected, AMF can dominate the phosphate supply to the plant (Smith et al., 2003, 2004), thereby benefiting the host plant without observable growth differences at the time of harvest. It has also been demonstrated that colonization ability can vary among AMF taxa (e.g., Douds et al., 1998; Graham and Abbott, 2000; Burleigh et al., 2002). When roots are colonized by more than one species of AMF, plants can uptake more phosphorus and exhibit greater plant growth than when colonized by a single AMF species (e.g., Jansa et al., 2008). Although we detected lower levels of AMF colonization in the *Bt* maize roots, we do not know if the *Bt* maize plants also had lower diversity of AMF taxa colonizing their roots. The local agricultural soil used in our study to inoculate the microcosms contained at least six different AMF taxa, so it is possible that, over time, one or a few more aggressive AMF species colonized the *Bt* roots (Graham and Abbott, 2000). More research, including molecular identification of the AMF taxa colonizing *Bt* and non-*Bt* maize roots, would help to determine whether *Bt* maize plants with lower levels of AMF colonization also have reduced diversity of AMF in their roots.

Historically, predictions of how different *Bt* plants may respond to AMF have been challenging because of the inconsistent results reported to date, even among *Bt* cultivars expressing the same protein. Complex interactions among soil organisms and the multitude of biotic and abiotic factors that contribute to mycorrhizal symbiosis in a given soil ecosystem have also been confounding factors in understanding the relationship between *Bt* plants and AMF. The complexity of the potential interactions of multiple types of *Bt* and non-*Bt* maize (e.g., herbicide-tolerance genes and gene products), on the responses of different maize lines to AMF infection were considered, however, previous studies have demonstrated little or no direct effect of the expression of herbicide-tolerance genes on soil microbes, AMF, or other soil fauna (e.g., Siciliano and Germida, 1999; Dunfield and Germida, 2003, 2004; Kowalchuk et al., 2003; Krogh et al., 2007; Griffiths et al., 2008; reviewed in Lundgren et al., 2009). Moreover, in our study, the parental control isolines that expressed

herbicide-tolerance genes had relatively high levels of AMF colonization in their roots, further indicating no direct effect of the expression on herbicide-tolerance genes on arbuscular mycorrhizae. Despite that we used only 10 replicates, and despite the variance that might influence AMF colonization in the different maize lines, our results demonstrated that AMF colonization was significantly lower in the *Bt* cultivars at both sampling dates. Many of the differences in colonization that were not significant may have been significant with a higher number of replicates, but this remains to be tested.

Mycorrhizal colonization has also been shown to vary within the same *Bt* maize line depending on fungal inoculum (species of AMF, mixed vs. pure cultures), the growth stage of the plant (early development, active growth, or reproductive stage), spore density, and fertilizer treatment (Cheeke et al., 2011). Because previous studies have evaluated AMF colonization in only one *Bt* plant line and under different experimental conditions, it has been difficult to compare the results among studies. Thus, maintaining the same environmental conditions throughout an experiment is critical for detecting the effects of different *Bt* maize cultivars on mycorrhizal fungi. To our knowledge, this study is the first demonstration of a reduction in AMF colonization across multiple *Bt* maize lines grown under the same experimental conditions. The use of endogenous mycorrhizal in whole soil inocula allowed each *Bt* and non-*Bt* maize line to interact with a community of soil organisms that might be expected under field conditions, making this study more ecologically relevant than other greenhouse studies where only pure spore cultures of one AMF taxa were used (e.g., Turrini et al., 2004; Castaldini et al., 2005; Cheeke et al., 2011). Future experiments should be conducted at the field level to verify the ecological significance of these findings and to examine whether long-term *Bt* crop cultivation has a negative effect on the abundance or diversity of AMF propagules in the soil ecosystem over time.

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Increasing Cropping System Diversity Balances Productivity, Profitability and Environmental Health

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Abstract

Balancing productivity, profitability, and environmental health is a key challenge for agricultural sustainability. Most crop production systems in the United States are characterized by low species and management diversity, high use of fossil energy and agrichemicals, and large negative impacts on the environment. We hypothesized that cropping system diversification would promote ecosystem services that would supplement, and eventually displace, synthetic external inputs used to maintain crop productivity. To test this, we conducted a field study from 2003–2011 in Iowa that included three contrasting systems varying in length of crop sequence and inputs. We compared a conventionally managed 2-yr rotation (maize-soybean) that received fertilizers and herbicides at rates comparable to those used on nearby farms with two more diverse cropping systems: a 3-yr rotation (maize-soybean-small grain + red clover) and a 4-yr rotation (maize-soybean-small grain + alfalfa-alfalfa) managed with lower synthetic N fertilizer and herbicide inputs and periodic applications of cattle manure. Grain yields, mass of harvested products, and profit in the more diverse systems were similar to, or greater than, those in the conventional system, despite reductions of agrichemical inputs. Weeds were suppressed effectively in all systems, but freshwater toxicity of the more diverse systems was two orders of magnitude lower than in the conventional system. Results of our study indicate that more diverse cropping systems can use small amounts of synthetic agrichemical inputs as powerful tools with which to tune, rather than drive, agroecosystem performance, while meeting or exceeding the performance of less diverse systems.

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Introduction

One of the key challenges of the 21st century is developing ways of producing sufficient amounts of food while protecting both environmental quality and the economic well-being of rural communities [1,2]. Over the last half century, conventional approaches to crop production have relied heavily on manufactured fertilizers and pesticides to increase yields, but they have also degraded water quality and posed threats to human health and wildlife [3–6]. Consequently, attention is now being directed toward the development of crop production systems with improved resource use efficiencies and more benign effects on the environment [1,7]. Less attention has been paid to developing better methods of pest management, especially for weeds. Here we explore the potential benefits of diversifying cropping systems as a means of controlling weed population dynamics while simultaneously enhancing other desirable agroecosystem processes [8]. We focus on crop rotation, an approach to cropping system diversification whereby different species are placed in the same field at different times.

Rotation systems have been used for millennia to maintain soil fertility and productivity and to suppress pests, and can increase yields even in situations where substantial amounts of fertilizers and pesticides are applied [9,10]. Rotation systems also foster spatial diversity, since different crops within the rotation sequence are typically grown in different fields on a farm in the same year. Diversification through crop rotation can be an especially useful strategy in farming systems that integrate crop and livestock production. The addition of forage crops, including turnips and clovers, to cereal-based systems in northwestern Europe and England in the 1600s and 1700s enhanced nitrogen supply through fixation by legumes, and increased nutrient cycling due to greater livestock density and manure production. These changes allowed the intensification of both crop and livestock production and increased yields substantially [11,12]. Integrated crop–livestock systems remained widespread in northern Europe, England, and much of the humid, temperate regions of North America until the 1950s and 1960s, when increased availability of relatively low-cost synthetic fertilizers made mixed farming and nutrient recycling biologically unnecessary and specialized crop and livestock production more economically attractive. In recent years, there has been interest in reintegrating crop and livestock systems as a strategy for reducing reliance on fossil fuels, minimizing the use of increasingly expensive fertilizers, and

limiting water pollution by nutrients, pathogens, and antibiotics [13,14].

Weeds are a ubiquitous and recurrent problem in essentially all crop production systems, and chemicals applied for weed control dominate the world market for pesticides [15]. With the introduction of crop genotypes engineered to tolerate herbicides, especially glyphosate, and with the continuing availability of older, relatively inexpensive herbicides, such as atrazine, successful weed management in conventional crop production systems has been largely taken for granted since the mid-1990s. Now, however, with expanded recognition of herbicides as environmental contaminants [4] and the increasing prevalence of herbicide resistant weeds [16], there is an important need to develop weed management strategies that are less reliant on herbicides and that subject weeds to a wide range of stress and mortality factors [17]. We believe that cropping system diversification may play an important role in the development of such strategies.

Here, we report the results of a large-scale, long-term experiment examining the consequences of cropping system diversification on agronomic, economic, and environmental measures of system performance. The experiment was conducted during 2003–2011 in Boone County, Iowa, within the central U.S. maize production region, and comprised three contrasting cropping systems varying in length of crop sequence, levels of chemical inputs, and use of manure. We compared a conventionally managed 2-yr rotation (maize-soybean) that received fertilizers and herbicides at rates comparable to those used on surrounding commercial farms with two more diverse cropping systems: a 3-yr rotation (maize-soybean-small grain + red clover) and a 4-yr rotation (maize-soybean-small grain + alfalfa-alfalfa) managed with reduced N fertilizer and herbicide inputs and periodic applications of composted cattle manure. Triticale was used as the small grain crop in 2003–2005; oat was used in 2006–2011. The 2-yr rotation is typical of cash grain farming systems in the region, whereas the 3-yr and 4-yr rotations are representative of farming systems in the region that include livestock. Details of the experimental site, management practices, sampling procedures, and data analyses are provided in the online SI section (Text S1, Figure S1, Tables S1-S4).

A central hypothesis framing our study was that cropping system diversification would result in the development of ecosystem services over time that would supplement, or eventually displace, the role of synthetic external inputs in maintaining crop productivity and profitability. Based on this hypothesis, we predicted that input requirements of the more diverse systems would initially be similar to that of the less diverse system, but would increasingly diverge from the less diverse system over time as the systems matured. We also predicted that crop yields, weed suppression, and economic performance of the three systems would be similar throughout the study. Finally, we predicted that reduced requirements for external synthetic inputs for pest management would result in a lower toxicological profile of the more diverse systems compared to the less diverse system.

Results

Crop Yields and Net Profitability

Cropping system diversification enhanced yields of maize and soybean grain and system-level harvested crop mass (grain, straw, and hay) while maintaining economic returns. The most parsimonious linear statistical models for each of these measures of system performance contained terms for main effects of *year* and *system*, but no interaction term ($AIC_{with\ interaction} = 319$; $AIC_{no\ interaction} = 315$). Over the 2003 to 2011 period, maize grain yield was on average 4% greater in the 3-yr and 4-yr rotations than in the 2-yr rotation (means for the 2-yr, 3-yr and 4-yr rotations are hereafter referred to as μ_2, μ_3 and μ_4, respectively; $\mu_2 = 12.3 \pm 0.1$ Mg ha^{-1}; $\mu_3 = 12.7 \pm 0.2$ Mg ha^{-1}; $\mu_4 = 12.9 \pm 0.2$ Mg ha^{-1}; pre-planned 1 d.f. contrast of *system*: $F_{1,7} = 8$, $P = 0.03$), and similar in the 3-yr and 4-yr rotations (Fig. 1a). Soybean grain yield during the same period was on average 9% greater in the 3-yr and 4-yr rotations than in the 2-yr rotation ($\mu_2 = 3.4 \pm 0.07$ Mg ha^{-1}; $\mu_3 = 3.8 \pm 0.08$ Mg ha^{-1}; $\mu_4 = 3.8 \pm 0.08$ Mg ha^{-1}; $F_{1,7} = 11.3$, $P = 0.01$) and similar in the 3-yr and 4-yr rotations (Fig. 1b). Harvested crop mass, averaged over the various crop phases comprising each cropping system, followed a similar pattern to maize and soybean grain yields. Mean crop biomass for 2003 to 2011 was 8% greater in the 3-yr and 4-yr rotations than in the 2-yr rotation ($\mu_2 = 7.9 \pm 0.08$ Mg ha^{-1}; $\mu_3 = 8.5 \pm 0.1$ Mg ha^{-1}; $\mu_4 = 8.6 \pm 0.2$ Mg ha^{-1}; *system*: $t_6 = 5.1$, $P = 0.002$), and similar in the 3-yr and 4-yr rotations (Fig. 1c).

We examined system profitability by calculating net returns to land and management, which represent profits to a farm operation without accounting for costs of land (e.g., rent or mortgage payments), management time (e.g., marketing), and federal subsidies. Profitability was analyzed for two temporal periods. From 2003 to 2005, considered the "startup" phase for the study, there were no differences among cropping systems in net profit, either through an analysis of main effects of *system* ($\mu_2 = \$448 \pm 17$ ha^{-1}; $\mu_3 = \$402 \pm 17$ ha^{-1}; $\mu_4 = \$457 \pm 15$ ha^{-1}; $F_{2,6} = 0.12$, $P = 0.89$) or by pre-planned 1-d.f. contrasts (2-yr vs. 3-yr and 4-yr rotations: $F_{1,7} = 0.10$, $P = 0.77$) (Fig. 1d). From 2006 to 2011, the "established" phase of the study, there were again no differences among systems, either through main effects of *system* ($\mu_2 = \$953 \pm 36$ ha^{-1}; $\mu_3 = \$965 \pm 34$ ha^{-1}; $\mu_4 = \$913 \pm 26$ ha^{-1}; $F_{2,6} = 0.62$, $P = 0.57$) or by pre-planned 1-d.f. contrasts (2-yr vs. 3-yr and 4-yr rotations: $F_{1,7} = 0.03$, $P = 0.86$).

Stability of system performance over time, as measured through a comparison of variances for the various products of the system, was similar for maize grain yield ($F_{2,6} = 2.4$, $P = 0.17$), soybean grain yield ($F_{2,6} = 0.95$, $P = 0.44$) and net returns to land and management during the startup phase of the study, 2003 to 2005 ($F_{2,6} = 0.05$, $P = 0.95$). Two system products, harvested crop mass from 2003 to 2011 and profit during the established phase of the study, 2006 to 2011, showed considerable differences in system stability over time, but in contrasting ways. Variance in mean harvested crop mass was greater in the 3-yr and 4-yr rotations than in the 2-yr rotation ($\sigma_2{}^2 = 0.27$; $\sigma_3{}^2 = 0.60$; $\sigma_4{}^2 = 0.95$; $F_{1,7} = 16$, $P = 0.005$). Conversely, cropping system diversification was associated with lower variance in profit during the established phase of the study. Variance in profit from 2006 to 2011 was lower in the 3-yr and 4-yr rotations than in the 2-yr rotation ($\sigma_2{}^2 = 1.5 \times 10^5$; $\sigma_3{}^2 = 8.1 \times 10^3$; $\sigma_4{}^2 = 6.3 \times 10^3$; $F_{1,7} = 16$, $P = 0.005$).

Agrichemical, Labor and Energy Inputs

Application rates of the primary agrichemicals used in this study, manufactured N fertilizer ($F_{2,14} = 117$, $P < 0.0001$) and herbicides ($F_{2,14} = 287$, $P < 0.0001$), both showed strong effects of cropping system. Manufactured N fertilizer applications were higher in the 2-yr rotation than in the 3-yr and 4-yr rotations ($\mu_2 = 80 \pm 3$ kg N ha^{-1}; $\mu_3 = 16 \pm 3$ kg N ha^{-1}; $\mu_4 = 11 \pm 2$ kg N ha^{-1}; $F_{1,17} = 16$, $P = 0.005$), with the difference between systems increasing over the course of the study ($F_{2,14} = 11.6$, $P = 0.001$) (Fig. 1e). Herbicide application rates followed a similar pattern, with greater amounts of herbicide applied in the 2-yr rotation than in the 3-yr and 4-yr rotations ($\mu_2 = 1.9 \pm 0.06$ kg a.i. ha^{-1};

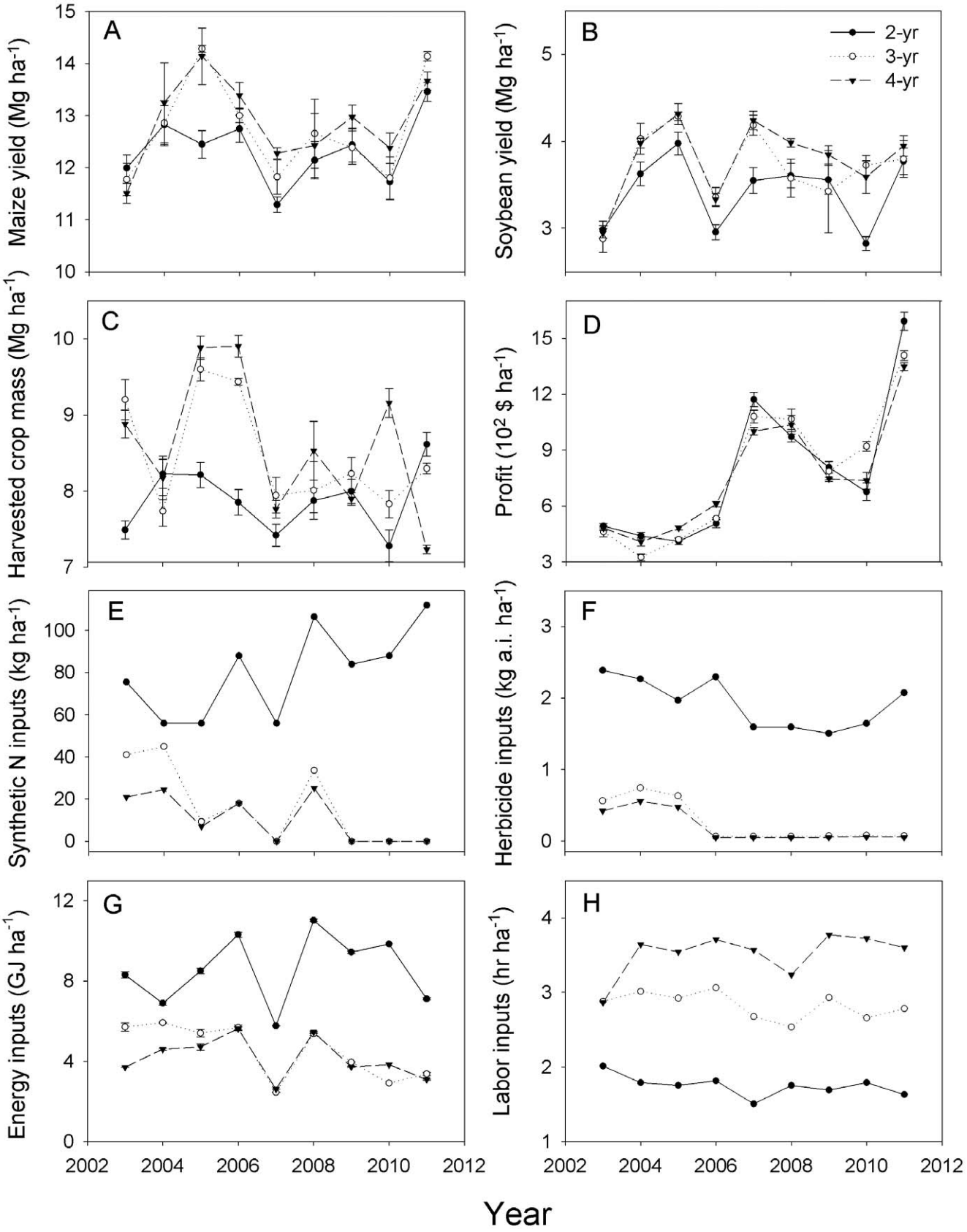

Figure 1. Cropping system performance over time. Annual performance of maize-soybean (2-yr), maize-soybean-small grain/red clover (3-yr), and maize-soybean-small grain/alfalfa-alfalfa (4-yr) cropping systems in Boone, IA, from 2003 to 2011. Performance metrics included: a) maize yield, b) soybean yield, c) rotation-level harvested crop mass, d) net returns to land and management, e) manufactured N fertilizer application rate, f) herbicide

application rate, g) fossil energy use, and h) labor requirements. Symbols represent the mean ± SEM of four replicate experimental blocks (N = 36 per cropping system).
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$\mu_3 = 0.26 \pm 0.05$ kg a.i. ha^{-1}; $\mu_4 = 0.20 \pm 0.03$ kg a.i. ha^{-1}; $F_{1,17} = 610$, $P < 0.0001$); differences among systems, however, did not increase over time (Fig. 1f).

Fossil energy use was strongly influenced by cropping system in both the startup ($F_{2,6} = 94$, $P < 0.0001$) and established ($F_{2,6} = 116$, $P < 0.0001$) phases of the study, with no difference in energy use between experimental phases ($F_{1,92} = 0.39$, $P = 0.53$) (Fig. 1g). From 2003 to 2011, inputs of energy were greater in the 2-yr rotation than in the 3-yr and 4-yr rotations ($\mu_2 = 8.6 \pm 0.1$ GJ ha^{-1}; $\mu_3 = 4.5 \pm 0.1$ GJ ha^{-1}; $\mu_4 = 4.2 \pm 0.04$ GJ ha^{-1}; $F_{1,7} = 55$, $P = 0.0001$). The partial correlations between energy use in a given cropping system and energy use in the maize phase of that rotation, taking into account the amount of N fertilizer applied to maize, were 0.94, 0.81 and 0.70 in the 2-yr, 3-yr and 4-yr systems, respectively (SI, Table S5). This indicated that synthetic N fertilizer use in the maize phase of the various cropping systems drove energy use within the maize phase, which in turn drove energy use by a given cropping system.

Demand for labor differed among the three cropping systems in both the startup ($F_{2,4} = 26$, $P = 0.005$) and established ($F_{2,10} = 299$, $P < 0.0001$) study phases, but followed a contrasting pattern to energy requirements (Fig. 1h). Labor inputs were more than 33% lower in the 2-yr rotation than in the 3-yr and 4-yr rotations from 2003 to 2005 ($F_{1,5} = 35$, $P = 0.002$) and from 2006 to 2011 ($F_{1,11} = 59$, $P < 0.0001$). Overall, there was a strong negative correlation ($r = -0.79$, $P < 0.0001$) between fossil energy and labor inputs over time in the three cropping systems.

Divergent Weed Management Systems

Two lines of evidence indicate that weeds were managed effectively in all three cropping systems in both the 'startup' and 'established' phases, in spite of reducing herbicide use by 88% in the 3-yr and 4-yr rotations compared to the 2-yr rotation. First, weed seedbanks declined at an equal rate in all study systems (Fig. 2a). Selection among linear mixed effects regression models incorporating temporal autocorrelation among seedbank measurements over time supported different intercepts (*system*: $F_{2,6} = 16.8$, $P = 0.0035$) but did not support inclusion of a *year* by *system* interaction term ($AIC_s = 182$; $AIC_{s*y} = 185$), thus indicating a common slope ($b_1 = -0.18$). For all three systems, the time to decline to 95% of the weed seedbank levels in 2003 was 16.6 years. Declines in weed seedbanks reflected a focus of management attention on the timing of weed management activities and herbicide choices in all three systems, as well as the increased number and diversity of stress and mortality factors present in the 3-yr and 4-yr rotations [8,21]. Higher densities of weed seeds in the 3-yr and 4-yr rotations, as indicated by their greater intercept values than for the 2-yr rotation (Fig. 2a.), were the result of poorer weed control in the 3-yr and 4-yr rotations during the set-up of the experiment plots in 2002.

The second line of evidence concerns weed biomass, which was very low in all three cropping systems for the duration of the study (Fig. 2b), never exceeding 0.3% of harvested crop mass. Weed biomass was the same within a given crop phase, regardless of the cropping system in which it occurred (main effect of *system*: maize, $F_{2,6} = 1.47$; $P = 0.30$; soybean, $F_{2,6} = 0.88$; $P = 0.46$; small grain, $F_{1,3} = 1.24$; $P = 0.31$). There were differences in mean weed biomass among cropping systems ($\mu_2 = 0.0003 \pm 0.00007$ Mg ha^{-1}; $\mu_3 = 0.0076 \pm 0.0012$ Mg ha^{-1}; $\mu_4 = 0.009 \pm 0.001$ Mg ha^{-1}; $F_{2,6} = 12.7$; $P < 0.007$). These differences arose mainly due

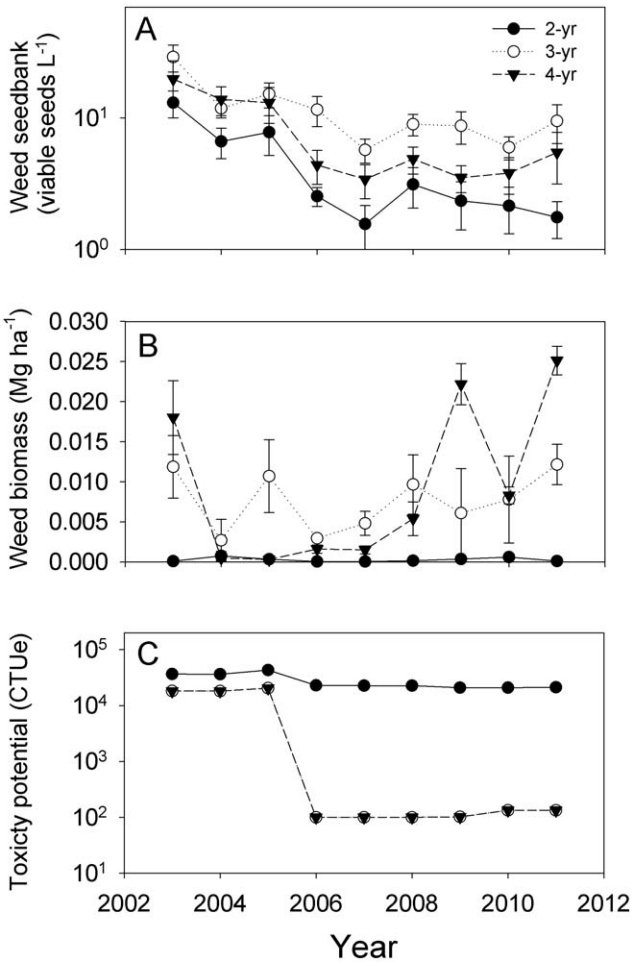

Figure 2. Divergent weed management systems. Weed management characteristics in maize-soybean (2-yr), maize-soybean-small grain/red clover (3-yr), and maize-soybean-small grain/alfalfa-alfalfa (4-yr) cropping systems in Boone, IA, from 2003 to 2011. Performance metrics included a) weed seed density in soil, b) weed aboveground biomass, and c) freshwater toxicity potential expressed in comparative toxic units (CTU_e). Symbols represent the mean ± SEM of four replicate experimental blocks (N = 36 per cropping system).
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to the presence of a small grain phase in the 3-yr and 4-yr rotation crop sequences. Weed biomass did not differ between maize and soybean in any of the cropping systems ($F_{1,202} = 2.1$; $P = 0.15$), however weed biomass in the small grain phase of the 3-yr and 4-yr rotations was greater than weed biomass in the maize and soybean phases ($F_{1,206} = 174$; $P < 0.0001$). In the 4-year system, weed biomass in alfalfa was intermediate between weed biomass levels in the maize/soybean and small grain phases.

Environmental toxicity, in relation to ecotoxicological profiles for herbicides used in this study (Fig. 2c), showed a strong effect of *system* ($F_{2,14} = 1673$, $P < 0.0001$), with lower toxicity potential in the 3-yr and 4-yr rotations compared to the 2-yr rotation (*type*: $F_{1,17} = 2691$, $P < 0.0001$). Ecotoxicity in the diversified and conventional systems diverged as the systems matured over time [*type* x *phase*: $F_{1,16} = 7.4$, $P = 0.015$], transitioning from a two-fold

difference during 2003 to 2005 to a two hundred-fold difference in toxicity from 2006 to 2011 (Fig. 2c).

Discussion

Our results support the hypothesis that the development of ecosystem services over time in more diverse cropping rotations increasingly displaces the need for external synthetic inputs to maintain crop productivity. From 2003 to 2011, as predicted, the desired products (crop yield, weed suppression, and economic performance) of the more diverse and less diverse cropping rotations were similar, whereas external inputs and environmental impacts differed greatly among the systems (Fig. 3). Comparing these metrics of system performance by experimental phase (initial three years of system establishment versus the following six years) confirmed our prediction that system inputs and environmental impacts would diverge over time, whereas yield and profit would remain similar among more diverse and less diverse rotations. In the more diverse rotations, small amounts of synthetic agrichemical inputs thus served as powerful tools with which to tune, rather than drive, agroecosystem performance.

Grain production in the U.S. is dominated by short rotation systems designed to maximize grain yield and profit. These are important goals but represent only a portion of the many ecosystem services that managed lands may provide [18] and that should be considered when evaluating alternative production systems [1,19]. We believe that these functions are complementary, rather than competing, considerations for agroecosystem design. The results of this study demonstrate that more rotationally diverse cropping systems may be optimized in multiple dimensions, leveraging small agrichemical inputs with biological synergies arising from enhanced diversity of crop species and management tactics.

An example of the synergizing effects of cropping system diversification can be found in weed management in the 3-yr and 4-yr rotations. Weeds were suppressed as effectively in these systems as in the 2-yr rotation, with declining soil seedbanks and negligible weed biomass, yet herbicide inputs in the 3-yr and 4-yr rotation plots were 6 to 10 times lower, and freshwater toxicity 200 times lower, than in the 2-yr rotation. Improved efficiency and environmental sustainability of weed management in the 3-yr and 4-yr rotations resulted from integrating multiple, complementary tactics in an ecological weed management framework [8,20]. Mounting evidence for unintended effects resulting from heavy reliance on herbicides highlights the need to re-think the role of herbicides in weed management. Non-target impacts of herbicides include reproductive abnormalities and mortality in vertebrates [5,21–23] and potential for diminished non-crop nectar resources for key pollinator species [17,24,25]. Herbicide overuse has also resulted in widespread, accelerating evolution of weed genotypes resistant to one or more modes of herbicide action [26,27]. Our

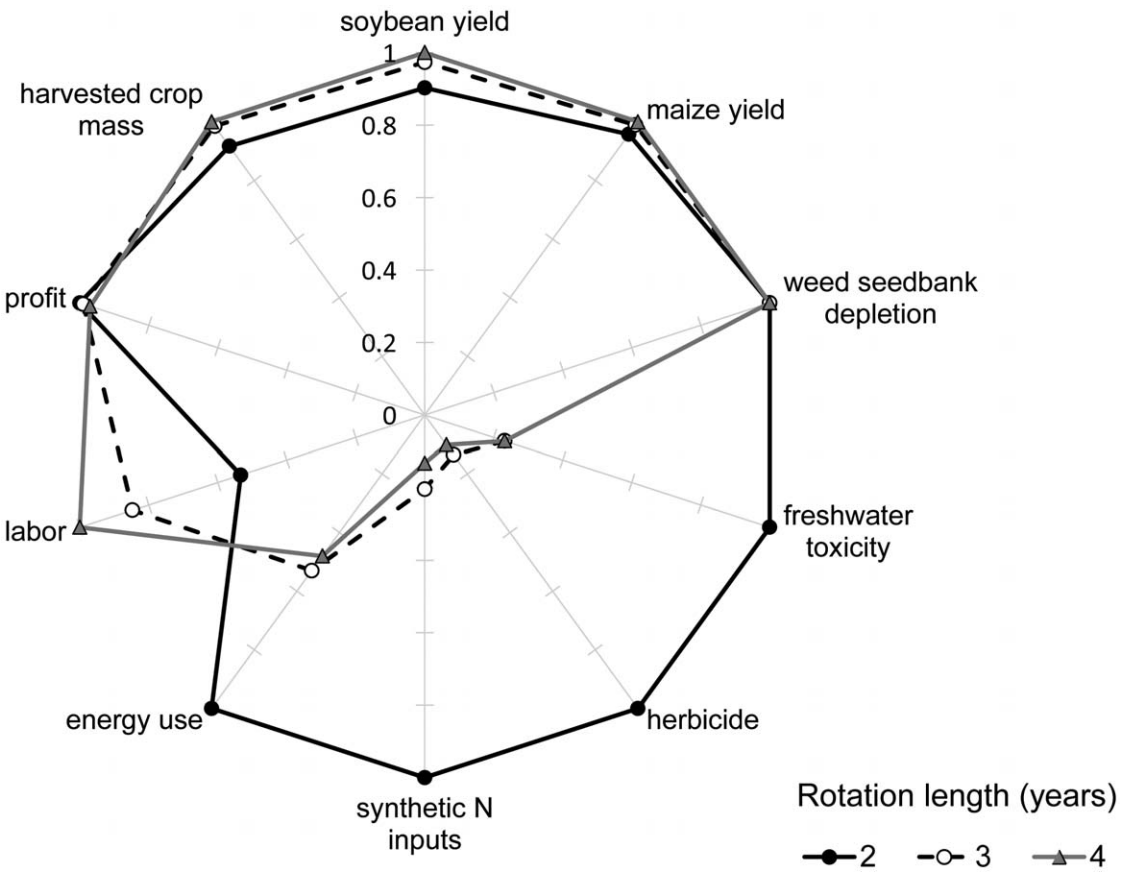

Figure 3. Multiple indicators of cropping system performance. Comparative long-term performance of maize-soybean (2-yr), maize-soybean-small grain/red clover (3-yr), and maize-soybean-small grain/alfalfa-alfalfa (4-yr) cropping systems in Boone, IA, averaged over the 2003–2011 study period. Variable means are normalized on a 0 to 1 scale, with 1 representing the cropping system with the largest absolute value for that variable (N = 36 per cropping system). Performance metrics included: maize and soybean yield, rotation-level harvested crop mass, net returns to land and management, manufactured N fertilizer and herbicide application rate, fossil energy use, labor requirements, freshwater toxicity potential and weed seedbank decline (measured as exponential decay constant).
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data indicate that, in the context of a cropping system with weed suppressive characteristics, small herbicide inputs may contribute to a diverse suite of tactics that cumulatively provide effective, reliable, and more durable weed management.

The diversity-productivity-stability relationship has long been a key theme in ecology [28,29]. Recently, it has been applied in the context of bioenergy crop production to describe increases in biomass and ecosystem services, such as C sequestration, associated with increasing species diversity in polycultures of bioenergy feedstock crop species [30]. Our work supports the application of this concept to cropping systems more broadly. Future gains in more diverse systems may depend upon the application of ecological principles surrounding this relationship to cropping system design [31,32]. Cropping system diversification in this study included both crop species and management practices. In contrast to the 2-yr rotation, with two species, both of the 3-yr and 4-yr rotations included four crop species. In the 4-yr rotation, further temporal diversification was achieved by including a perennial-only crop phase (alfalfa hay) for one quarter of the rotation sequence. Our results showed productivity gains associated with greater diversity in system-level harvested crop mass and maize and soybean seed yields. We also observed increased stability of profit, with similar long-term means, in the 3-yr and 4-yr rotations compared to the 2-yr rotation.

Similar profits were attained through different pathways in the 3-yr and 4-yr rotations and the 2-yr rotation (Fig. 3). Increased labor, information intensive management and ecosystem services arising from increased biological N fixation (via the clover and alfalfa crops) and contrasting crop phenologies and competitive abilities were substituted in 3-yr and 4-yr rotations for the higher inputs of manufactured N, herbicides and energy from fossil fuels driving the 2-yr rotation. Energy use in maize drove differences among the cropping systems, and manufactured N inputs to maize contributed most strongly to energy balances for this crop. The high sensitivity of agricultural energy use to N fertilizer inputs provides a high-priority target for the redesign of cropping systems for increased sustainability.

Reintegration of crop and livestock production, as represented by the forage legumes and manure applications present in the more diverse systems, is not simply another aspect of cropping system diversification. Instead, it embodies an important principle in sustainable agriculture: system boundaries should be drawn to minimize externalities. Animal manure is produced regardless of whether feed grains are shipped to centralized concentrated animal feeding operations, or produced within integrated crop-livestock farming operations. In the former case, the manure may become a waste product and water pollutant if quantities exceed available land area for field application [33], whereas in the latter case, it contributes directly to crop nutrient requirements, improves soil quality, and reduces fossil fuel subsidies associated with grain transport and external N fertilizer inputs [14].

Substantial improvements in the environmental sustainability of agriculture are achievable now, without sacrificing food production or farmer livelihoods. When agrichemical inputs are completely eliminated, yield gaps may exist between conventional and alternative systems [19]. However, such yield gaps may be overcome through the strategic application of very low inputs of agrichemicals in the context of more diverse cropping systems. Although maize is grown less frequently in the 3-yr and 4-yr rotations than in the 2-yr rotation, this will not compromise the ability of such systems to contribute to the global food supply, given the relatively low contribution of maize and soybean production to direct human consumption and the ability of livestock to consume small grains and forages [34]. Through a balanced portfolio approach to agricultural sustainability, cropping system performance can be optimized in multiple dimensions, including food and biomass production, profit, energy use, pest management, and environmental impacts.

Materials and Methods

Site Details and Agronomic Management

To investigate the relative performance of conventional and more diverse cropping systems, we conducted a 9-hectare experiment at the Iowa State University Marsden Farm (Figure S1), in Boone County, IA (42°01′ N; 93°47′ W; 333 m above sea level). The experiment site lies within a region of intensive rain-fed maize and soybean production and is surrounded by farms with high levels of productivity. Soils at the site are deep, fertile Mollisols. The experimental cropping system treatments included a conventionally managed 2-yr rotation (maize/soybean) that received agrichemicals at rates comparable to those used on commercial farms in the region, and more diverse cropping systems – a 3-yr rotation (maize/soybean/small grain + red clover green manure) and a 4-yr rotation (maize/soybean/small grain + alfalfa/alfalfa hay) – managed with reduced N fertilizer and herbicide inputs.

The entire site was planted with oat in 2001 and the cropping systems experiment was established in 2002 using a randomized complete block design with each crop phase of each rotation system present every year in four replicate blocks. Plots were 18 m x 85 m and managed with conventional farm machinery. Spring triticale was used as the small grain in 2003–2005, whereas oat was used in 2006–2010. Synthetic fertilizers were applied in the 2-yr rotation at conventional rates based on soil tests. In the 3-yr and 4-yr rotations, composted cattle manure was applied before maize production at a mean dry matter rate of 8.3 Mg ha^{-1} and substantial amounts of N were added through fixation by red clover and alfalfa [35,36,37]. Manure and legume N-fixation in the 3-yr and 4-yr rotations were supplemented with synthetic fertilizers based on soil tests, including the late-spring soil nitrate test for maize production [38]. Weed management in the 2-yr rotation was based largely on herbicides applied at conventional rates. In the 3-yr and 4-yr rotations, herbicides were applied in 38-cm-wide bands in maize and soybean and inter-row zones were cultivated; no herbicides were applied in small grain and forage legume crops. Choices of post-emergence herbicides used in each of the systems were made based on the identities, densities, and sizes of weed species observed in the plots. Other details of the farming practices used in the different cropping systems are described in Liebman et al. [39] and in the online SI materials (Text S1). Sampling procedures for determining crop yields, weed biomass and weed seed densities in soil are also described in the online SI materials (Text S1).

Energy and Economic Analyses

Energy inputs were divided into five categories: seed, fertilizer, pesticides, fuel for field operations, and propane and electricity used for drying maize grain after harvest. Data were obtained from logs describing all field operations, material inputs, and crop moisture characteristics for the experimental plots during the study period. Economic analyses measured performance characteristics of whole rotation systems under contrasting management strategies. We evaluated net returns to land and management on a unit land area basis, with land units divided in two equal portions for maize and soybean in the 2-yr rotation; three equal portions for maize, soybean, and small grains with red clover in the 3-yr rotation; and four equal portions for maize, soybean, small grains with alfalfa, and alfalfa in the 4-yr rotation. Net returns to land and management represented returns to a farm operation calculated

without accounting for costs of land (e.g., rent or mortgage payments), management time (e.g., marketing), or possible federal subsidies. Data sources and assumptions for the energy and economic analyses are shown in the online SI materials.

Ecotoxicological Calculations

Freshwater ecotoxicity of pesticide use was estimated using the USEtox model [40–42]. Characterization factors (CFs) of ecotoxicity potential for active ingredients included transport to freshwater via surface water, soil, and air. CFs were available for eight of ten active ingredients applied in the three rotations. The two active ingredients for which CFs were unavailable are not of particular concern for freshwater ecotoxicity due either to their low toxicity (mesotrione) or low infiltration and persistence in freshwaters (lactofen) [43].

Statistical Analyses

The experiment was arranged in a randomized complete block design, with all entry points of the three crop rotations (i.e. all crops within each of the rotations) represented in four replicate blocks in each year of the study, for a total of 36 plots. Cropping system effects in time series data were analyzed using hierarchical linear mixed effects repeated measures models, modeling temporally correlated errors with an ARMA (auto-regressive moving average) correlation structure in the *nlme* package of R v.2.14.1 [44,45]. Fixed effects included *cropping system* and *experimental phase* (startup = 2003 to 2005; established = 2006 to 2011), and random effects included *replicate block* nested within *cropping system* and *year*. Partial correlations were estimated using the *corpcor* package in R v.2.14.1. In contrast to data for quantitative observations (e.g. crop yield or weed biomass) that varied by replicate block and year, data for input variables, such as synthetic fertilizer or herbicides and associated environmental toxicity metrics, did not vary among blocks for a particular rotation entry point in a given year, but did vary among years. Therefore, site-year was treated as the source of experimental replication for these latter variables in our statistical tests for effects of *cropping system* and *experimental phase*. This led to contrasting degrees of freedom in reported F-tests for these two data types. Finally, for variables with non-constant variance among cropping systems over time (crop biomass and profit), we used the 'varIdent' variance function within the *nlme* package to explicitly model differences in variances among cropping systems for these variables within our mixed effects models.

Supporting Information

Figure S1 Aerial view of Marsden Farm study, Boone IA. Crop abbreviations: m = maize, sb = soybean, g = small grain, a = alfalfa.
(TIF)

Table S1 Mean monthly air temperature and total monthly precipitation during the 2003–2011 growing seasons, and long-term temperature and precipitation averages. Data were collected about 1 km from the experimental site in Boone Co., IA.
(DOCX)

Table S2 Crop identities and seeding rates in 2003–2011.
(DOCX)

Table S3 Macronutrients applied in manufactured fertilizers, herbicide adjuvants, and manure in 2003–2011. Manufactured N, P, and K fertilizers were applied at rates that varied among years and rotations in response to soil test results. Manure was applied at a rate of 15.7 Mg ha^{-1} in maize phases of the 3-year and 4-year rotation systems, but moisture and nutrient concentrations varied among years, resulting in variable rates of macronutrient additions.
(DOCX)

Table S4 Herbicide applications in 2003–2011 to maize and soybean in the three rotation systems. No herbicides were used for triticale, oat, red clover, and alfalfa grown within the 3-yr and 4-yr systems. Reported application rates reflect the effect of banding of herbicides over crop rows in the 3-yr and 4-yr systems.
(DOCX)

Table S5 Simple and partial correlations between energy use within a given crop phase and mean rotation energy use and between energy use within a given crop phase and N fertilizer application rates.
(DOCX)

Text S1 Detailed description of experimental site, management practices, scientific methods and statistical approach.
(DOCX)

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Author Contributions

Conceived and designed the experiments: ASD ML. Performed the experiments: CAC AMJ ML. Analyzed the data: ASD JDH CAC AMJ ML. Contributed reagents/materials/analysis tools: ML. Wrote the paper: ASD JDH ML.

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Environmental Concerns with the Development of Herbicide-Tolerant Plants[1]

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Abstract. Development of herbicide-tolerant plants is the focus of considerable research. Some projects aim to increase herbicide use or promote use of particularly environmentally damaging chemicals, and thus may lead to environmental degradation. Other projects aim to develop herbicide-tolerant plants that allow substitution of newer less environmentally damaging chemicals for older more damaging ones. To the extent they divert research dollars from development of other weed control strategies, these projects may also jeopardize environmentally sound weed control. The paper concludes with policy recommendations concerning a) public sector research priorities, b) planting of herbicide-tolerant trees in forests, and c) regulation of herbicide-tolerant crops.
Additional index words: Forestry, food safety, research priorities.

INTRODUCTION

In the mid-1980's, some proponents of biotechnology touted this rapidly emerging field as the solution to a number of pressing environmental problems, including those caused by agricultural chemicals. They said the products of agricultural biotechnology soon would provide substitutes, and thus reduce, if not eliminate, the use of many hazardous agricultural chemicals (1, 9, 22). Largely because of these promises, many in the "alternative" agriculture and environmental communities feel a sense of betrayal (6, 19, 21). Instead of providing substitutes for agrichemicals, some of the first major products of agricultural biotechnology likely will be plants genetically modified specifically to promote use of synthetic chemical herbicides.

In contrast, a number of weed scientists and other agriculturalists have argued that development of herbicide-tolerant plants is consistent with the goal of using biotechnology to solve environmental problems. They claim that herbicide-tolerant crops will cause farmers to substitute certain newer, less environmentally damaging herbicides for older, more damaging chemicals (2, 20, 31, 35).

The purpose of this paper is to examine these seemingly opposing views from the perspective of an environmentalist. To do this, I will examine the extent of research to develop herbicide-tolerant plants, the environmental effects of herbicides, and the environmental consequences of developing herbicide-tolerant plants. I will conclude by making several public policy recommendations concerning herbicide-tolerant plants.

EXTENT OF RESEARCH TO DEVELOP HERBICIDE-TOLERANT PLANTS

As a proportion of all pesticides used in the United States, herbicide use has grown tremendously over the past two decades. By weight of active ingredients, herbicides now constitute roughly 60% of all pesticides used (43) and 85% of all pesticides applied to field crops (38).

Particularly because herbicides now dominate the pesticide market, agrichemical companies have tremendous financial incentive to promote sales of herbicides they manufacture. Most major agrichemical firms now own seed companies (6), and sale of seed for herbicide-tolerant plants presents one mechanism of promoting herbicide sales. Industry estimates vary, but a number of analysts believe that the availability of herbicide-tolerant crops could boost annual sales of particular herbicides by more than $100 million (13).

Given this clear incentive, and recent scientific advancements that make herbicide tolerance relatively straightforward to achieve (20, 31), development of herbicide-tolerant crops has become a major focus of commercial biotechnology research. A recent survey of reports published between 1985 and 1989 in trade journals, newsletters, and other publications indicates that at least 27 corporations, including the world's eight largest pesticide manufacturers, have initiated research to develop herbicide-tolerant crops (13). It should be noted that the accuracy of such a survey is somewhat limited. In particular, some firms may have terminated projects, while research by others may go unreported.

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Permits granted by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS)[3] for field tests of plants modified by recombinant DNA techniques are another indication of the extent of research to develop herbicide-tolerant crops. As of Nov. 16, 1990, 35 of the 96 permits granted by APHIS were for field tests of herbicide-tolerant crops.[4] All 35 of these permits were to private firms.

Development of herbicide-tolerant crops is not, however, limited to the private sector. In 1989, a USDA committee declared development of herbicide-tolerant plants a research priority (40). USDA and state governments are funding considerable research to develop herbicide-tolerant plants, although the number of projects is disputed. One computer search of USDA's Current Research Information System, revealed 409 project entries for publicly funded research in 1989 concerning herbicide-tolerance (13). Of these, 58 projects stated as an objective or accomplishment the use of genetic manipulation to achieve herbicide-tolerance in plants. Another survey found 219 project entries concerning herbicide-tolerance in 1990, 21 of them had as an objective or an accomplishment the use of genetic manipulation to achieve herbicide-tolerance in plants (8).

ENVIRONMENTAL EFFECTS OF HERBICIDES

The sheer magnitude of herbicide use–roughly 293 × 10^6 kg of herbicide active ingredients were applied in 1987 (43)–along with the new emphasis on development of herbicide-tolerant crops, has sparked new scrutiny of herbicides by environmentalists.

One might expect that herbicides would present little risk to humans and other vertebrates, because the physiology and morphology of plants and animals differ significantly. Indeed, many herbicides exhibit little acute toxicity to vertebrates. Nevertheless, some herbicides have caused fatal poisonings of humans (26).

Although the extent of epidemiological studies and chronic toxicity data for many herbicides is limited, over the years a number of herbicides have been implicated as chronic toxins (26, 27, 30). Exposure to certain herbicides is linked with birth defects, skin disease, nervous system disorders, and cancer. The U.S. Environmental Protection Agency (EPA)[3], for example, lists acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid}, alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide], and lactofen {(±)-2-ethoxy-1-methyl-2-oxoethyl 5[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate} as probable human carcinogens, and atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine], linuron [*N*′-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea], and trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine], among others, as possible human carcinogens (44).

In the face of such evidence, a number of individuals have expressed considerable concern about residues of pesticides, including herbicides, on food (25, 27) The National Research Council (27) estimates that herbicides account for 31% of the oncogenic risk of pesticide residues on fresh food.

The presence of agricultural chemicals in drinking water has also aroused considerable concern. Until the late 1970's, most people thought that soil and rock formations protected groundwater from contamination by pesticides (45). But this belief has rapidly changed: As of 1988, EPA had detected 74 pesticides in the groundwater of 38 states. Of these, 21 were detections of herbicides that the agency could confirm as due to normal agricultural use (47). Although most of these detections were at low concentrations, some detections of herbicides were an order of magnitude above EPA's health advisory level for those chemicals.

Groundwater contamination by pesticides is extremely troubling. About 97% of the drinking water for rural populations, and half the drinking water for the entire U.S. population, comes from groundwater (42). And, once contaminated, groundwater is extremely difficult and expensive to clean up. Moreover, because groundwater is typically slow to recharge, contamination likely could remain for years even after remedial action is taken (30). This contrasts with the risks associated with pesticide residues on food, which could be lessened in a growing season by appropriate changes in pest control practices.

Herbicide use may also cause other adverse environmental effects not discussed here, including surface water contamination, occupational risks to farmers and

[3]Abbreviations: APHIS, Animal and Plant Health Inspection Service; EPA, U.S. Environmental Protection Agency; FIFRA, Federal Insecticide, Fungicide, and Rodenticide Act.

[4]U.S. Dep. Agric., Animal and Plant Health Inspect. Serv. Permits issued for release into the environment under 7 CFR 340.

farmworkers, and hazards to wild plants and animals (13).

ENVIRONMENTAL CONSEQUENCES OF HERBICIDE-TOLERANT PLANTS

Will the development of herbicide-tolerant plants exacerbate these already pressing environmental problems? A quick answer is that they may. But, the environmental consequences of the development of herbicide-tolerant plants will vary. They will depend on which plants are genetically modified to tolerate herbicides, which herbicides they are modified to tolerate, and, for crops that are raised for human or animal consumption, the safety of herbicide-tolerant plants as food.

Plants being modified. Whether or not herbicide-tolerant plants increase the amount of crop land treated with herbicides will depend on whether particular crops are already heavily treated. Plants being modified to tolerate herbicides range from field crops, such as corn (*Zea mays* L.), soybeans (*Glycine max* (L.) Merr.), and cotton (*Gossypium hirsutum* L.), to forest trees such as poplar (*Populas* ssp.)[4] (13).

Herbicide use on many field crops is already very high; in 1988, 95% of U.S. cotton acreage and 96% of U.S. corn and soybean acreage were treated with herbicides (38). Thus the availability of herbicide-tolerant field crops cannot lead to a large increase in the proportion of acreage treated with herbicides. The availability of herbicide-tolerant crops could, however, influence the frequency and amount of herbicide applications. Changes will vary by crop, region, and herbicide.

In contrast, only a small fraction of forest acres are sprayed aerially with herbicides, although the proportion varies with forest ownership, terrain, and tree species harvested. (Unwanted trees are also sometimes directly injected with herbicide.) Arguing that other weed control measures are expensive, U.S. Forest Service researchers are developing herbicide-tolerant trees explicitly to increase herbicide use in forests[5] (29, 39).

In short, development of some herbicide-tolerant plants, such as a number of major field crops, is unlikely to significantly increase acreage treated with herbicides. Development of other herbicide-tolerant plants, such as forest trees, will promote increased use of herbicides–and whatever adverse environmental effects accompany increased use.

Encouraging herbicide use in forests also presents special management issues, as forests are typically used for conservation and recreation, as well as timber production. Herbicide applications may pose hazards to fish and wildlife populations, and certainly alter plant community composition. Intensive application of herbicides to suppress pioneer vegetation after clear-cutting can also lead to soil erosion and nutrient loss (4). In short, herbicide-tolerant forest trees may make short term economic sense for foresters, but they are generally incompatible with land stewardship. Using them in government forests would be strong expression of timber primacy–the idea that our national and state forests are managed primarily for timber production, rather than conservation and recreation.

Targeted herbicides. Proponents of herbicide-tolerant plants commonly argue that these plants will promote the use of relatively new and environmentally benign herbicides. Herbicides that plants are being modified to tolerate include newer chemicals such as glufosinate [2-amino-4(hydroxymethylphosphinyl)butanoic acid], glyphosate [*N*-(phosphonomethyl)glycine], sethoxydim {2-[1-(ethoxyimino)butyl)-5-(2-(ethylthio)propyl]-3-hydroxy-2-cyclohexene-1-one}, and various imidazolinones and sulfonylureas[4] (13).

Not all research, however, it directed toward such chemicals. Plants are also being modified to tolerate older chemicals such as bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), atrazine, metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5(4*H*)-one], and 2,4-D [(2,4-dichlorophenoxy)acetic acid][4] (13, 17, 33). Bromoxynil is absorbed dermally, and because it causes birth defects in rodents, likely poses a hazard to farmers and farmworkers.[6] It is also highly toxic to fish (18), and in 1989, EPA declared bromoxynil a restricted use pesticide.[7] Atrazine, which EPA lists as a possible carcinogen, has been detected in groundwater across the country (44, 47). Metribuzin and 2,4-D both leach into groundwater from normal agricultural use (47), and a number of epidemiological studies (but not all) suggest that 2,4-D is a carcinogen (15, 46, 48).

[5]Haissig, B. E. 1984. Genetic transformation of forest trees in microculture. Research unit description FS-NC-1403. U.S. Dep. Agric. For. Serv. North Cent. For. Exp. Stn., Rhinelander, WI.

[6]Agric. Canada, Pesticides Directorate. June 2, 1989. CAPCO Note, Bromoxynil; U.S. Environ. Prot. Agency, Office of Pesticides and Toxic Substances. February 1990. Suspended, Canceled, and Restricted Pesticides.

[7]U.S. Environ. Prot. Agency, Office of Public Affairs. May 9, 1989. Environmental News: EPA Imposes Risk Reduction Measures for Bromoxynil Pesticide.

Benbrook and Moses (3) estimate that roughly 25% of research to develop herbicide-tolerant plants is targeted to older compounds. Promoting use of such herbicides by developing herbicide-tolerant plants will perpetuate, if not increase, the environmental problems caused by these herbicides.

A large fraction of research to develop herbicide-tolerant plants, however, focuses on newer herbicides. Available evidence indicates that these chemicals are less environmentally damaging then many older herbicides. Substituting them for older herbicides arguably results in an immediate net benefit to the environment.

Nevertheless, formulations of these chemicals, as applied by farmers, are not free from environmentally damaging characteristics. Poast,[8] a commercially available formulation of sethoxydim, is 18% xylene and 5% napthalene[9] –both of which are acute toxins (23, 32). Glyphosate formulations are one of the leading causes of skin irritations, according to reports by California farmworkers (24), and are somewhat toxic to fish fry (10). The highly potent sulfonylureas can have disastrous consequences if they drift onto adjacent crops and native vegetation (36).

The newer herbicides may not always be good substitutes for older chemicals. Weeds have rapidly evolved resistance to some sulfonylurea herbicides, and many agriculturalists now recommend these chemicals be mixed with older herbicides such as 2,4-D (5, 11). Furthermore, widespread availability of crops tolerant to the newer herbicides, and a concomitant increase in use of the newer herbicides, could lead to increased incidence of weeds that resist these herbicides and decreased usefulness of these herbicides to farmers.

In short, the development of crops tolerant to the newer herbicides cannot be regarded as a panacea for current environmental problems resulting from herbicide use. Decisions to rely on the newer herbicides for weed control must be weighed against the relative environmental safety and long term benefits of weed management strategies based on measures such as crop rotation, ridge tillage, cover crops, and when available, biological controls (12, 14, 16, 28, 37). Adoption of such weed management strategies would likely be more widespread if weed scientists had more resources to devote to their development. To the extent that development of herbicide-tolerant crops diverts resources from such research, these crops jeopardize environmentally sound weed control.

Food safety. Because most crops are grown as food for humans or feed for livestock, most herbicide-tolerant plants will have to be safe for consumption by vertebrates. It is difficult to imagine gene products that confer herbicide tolerance as posing a risk to consumers. But, as illustrated by the following two examples, both the degradation products and accumulation of herbicides in tolerant plants need to be considered before plants can be accepted as safe.

First, 2,4-D resistance can be achieved by transforming plants with a gene coding the enzyme that catalyzes the first step in the bacterial 2,4-D degradative pathway (34). Degradation of 2,4-D results in the formation of 2,4-dichlorophenol (2,4-DCP), a chemical that EPA and the Agency for Toxic Substances and Disease Registry have listed as a hazardous substance under the Superfund Amendments and Reauthorization Act of 1986. In laboratory animals ingestion of 2,4-DCP causes liver damage, skin sores, and at high concentrations, death (41). Thus, 2,4-D tolerant plants may not be acceptable for human consumption following treatment with 2,4-D, depending on whether 2,4-DCP is rapidly degraded in plant tissue.

Second, glyphosate is readily absorbed and translocated by vascular plants (7). This herbicide is known to accumulate in storage organs such as fruits and tubers, and evidence suggests that glyphosate is subject to little metabolic degradation in plant tissues. Thus it is reasonable to speculate that glyphosate-tolerant crops treated with this herbicide might accumulate glyphosate in their harvested tissues. This possibility, and its implications for food safety, need to be carefully evaluated before glyphosate-tolerant crops are marketed as foods.

CONCLUSION AND POLICY RECOMMENDATIONS

This paper reports that considerable private and public sector research is focused on the development of herbicide-tolerant plants, and that current widespread use of herbicides significantly contributes to environmental degradation. The development of herbicide-tolerant plants may, in a number of instances, promote increased herbicide use or use of particularly environmentally damaging chemicals. In the special case of forestry, herbicide-tolerant trees would encourage chemical use inappropriate in areas conserved as natural ecosystems.

[8]BASF Corp., P.O. Box 13528, Research Triangle Park, NC 27709.
[9]BASF. 1986. Material Safety Data Sheet for Poast.

In other instances, herbicide-tolerant plants may allow farmers to substitute newer less environmentally damaging chemicals for older more environmentally damaging chemicals used on crops already heavily treated with herbicides. Nevertheless, although the environmental problems associated with many newer herbicides are less severe than those associated with many older herbicides, newer herbicides are not free of environmental problems. Moreover, resistant weeds already limit use of some of the newer chemicals, and the availability of crops that tolerate the newer herbicides could further encourage the evolution of resistant weeds. Thus, substitutions of newer for older herbicides via herbicide-tolerant plants may provide some immediate environmental benefits, but simply switching chemicals is not the long term path toward environmentally benign weed control.

Some herbicide-tolerant crops may also be of questionable safety for human or animal consumption, implying that premarket review of herbicide-tolerant crops will be necessary to protect public health.

From these conclusions I make the following public policy recommendations:

Public sector research priorities. Development of herbicide-tolerant plants should not be a focus, as it currently is, of public sector research. As many companies have illustrated by their choices of research projects, there is a clear profit incentive for private sector development of herbicide-tolerant plants. Public sector researchers should not try to duplicate this effort, especially in an era when research funds are scarce. Rather, taxpayer-supported researchers should focus on developing weed control practices and products that are not profitable for the private sector to develop, but potentially benefit farmers and members of society at large. Options besides chemical weed control, and management programs to minimize herbicide use by farmers, exemplify appropriate research focuses. Because there are few other avenues for pursuit of such research, many environmentally sound approaches to weed control will remain underdeveloped if public sector researchers do not make them a priority.

Herbicide-tolerant trees. In addition to not funding research to develop herbicide-tolerant trees, the U.S. Forest Service and other government agencies should establish a policy of not introducing herbicide-tolerant trees to national forests and other government lands. As discussed above, the increased use of herbicides that would result from the planting of herbicide-tolerant trees is generally incompatible with the multiple-uses that should be the objectives of most government forest management.

It is especially ironic that the Forest Service is developing herbicide-tolerant trees at the same time as a lawsuit settlement[10] has forced the Pacific Northwest management region of the Forest Service and the northwest office of the Bureau of Land Management to prepare environmental impact statements that consider the effects of vegetation management practices on natural ecosystems, as well as timber production. The Forest Service's final impact statement promotes the reduction of herbicide use as the preferred alternative for vegetation management.

Regulation of herbicide-tolerant plants. Plants genetically modified to tolerate herbicides are currently not regulated for their impacts on herbicide use nor do they generally receive premarket approval for their safety as foods. This is not surprising, because prior to the development of modern techniques for genetic manipulation, genetic modification of plants did not typically allow the rapid introduction of new tolerance mechanisms to plants nor result in potentially dramatic changes in herbicide use.

To accommodate these technological changes, two new regulatory policies should be adopted for plants genetically engineered to tolerate herbicides and intended for commercial use. First, the EPA should regulate herbicide-tolerant plants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)[3]. EPA should restrict the use herbicide-tolerant plants if they promote environmental degradation by increased use of hazardous chemicals (or by other means not discussed in this paper, such as transfer via cross-pollination of herbicide-tolerance genes to weeds (13)). Second EPA and the Food and Drug Administration should regulate herbicide-tolerant plants intended for human and animal consumption under FIFRA and the Federal Food, Drug and Cosmetic Act. Plants should not be marketed if herbicide residues–including sequestration and degradation products–pose a significant health risk.

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[10]Northwest Coalition for Alternatives to Pesticides v. Block, Civil No. 83-6272-E-BU (D. Ore. 1984).

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The Establishment of Genetically Engineered Canola Populations in the U.S.

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Abstract

Concerns regarding the commercial release of genetically engineered (GE) crops include naturalization, introgression to sexually compatible relatives and the transfer of beneficial traits to native and weedy species through hybridization. To date there have been few documented reports of escape leading some researchers to question the environmental risks of biotech products. In this study we conducted a systematic roadside survey of canola (*Brassica napus)* populations growing outside of cultivation in North Dakota, USA, the dominant canola growing region in the U.S. We document the presence of two escaped, transgenic genotypes, as well as non-GE canola, and provide evidence of novel combinations of transgenic forms in the wild. Our results demonstrate that feral populations are large and widespread. Moreover, flowering times of escaped populations, as well as the fertile condition of the majority of collections suggest that these populations are established and persistent outside of cultivation.

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Introduction

Crop and forage species now cover more than one quarter of the Earth's land surface [1], but the ecological and evolutionary influences of agricultural species on native and weedy plants have been difficult to measure. The commercial release of GE crops has provided novel genetic markers to track crop-to-weed gene flow [2,3] raising both awareness of the difficulties of transgene confinement and concerns about the ecological consequences of transgenes in the environment [4,5]. Genetically engineered varieties could influence the population ecology of wild species by introducing novel, beneficial traits, or lead to detrimental effects such as extirpation of native alleles or declines of natural populations [6]. The escape of crops or crop alleles is no longer in doubt [7], but reports of transgene escape are few and are limited in the U.S. to the case of creeping bentgrass, *Agrostis stolonifera* (Poaceae), from a field trial in central Oregon, USA [8,9]. Given that biotech crops cover more than 130Mha globally [10], the rarity of reported escapes has led some to question the environmental risks of genetically engineered crops [11,12].

Canola (*Brassica napus* L. (Brassicaceae)) is an oilseed crop grown on approximately 31Mha globally [13]. *Brassica napus*, an allotetraploid formed by the hybridization of *B. rapa* L. and *B. oleraceae* L., is sexually compatible with more than 15 other mustard species [14], a number of which are considered noxious weeds [15]. Canola cultivars engineered for glyphosate and glufosinate herbicide resistance escaped cultivation shortly after their unconditional commercial release in Canada in 1995 [16] and more recent research has documented widespread escape and persistence of transgenic canola in Canadian roadside populations [17,18]. Since these discoveries, feral canola populations or non-engineered populations expressing biotech traits have been reported from Great Britain, France, Australia and Japan [2,3,19–21]. In the U.S., GE canola was first approved for commercial release in 1998 and now most (>90%) of the acreage planted in the U.S. is genetically engineered for herbicide resistance [10].

The objective of this study was to document the extent of feral canola populations in North Dakota, the dominant canola growing region of the United States. We used roadside surveys and commercially available test strips evaluate the distribution of transgenic canola growing outside of cultivation in the U.S.

Materials and Methods

We conducted systematic roadside surveys to quantify the presence and abundance of feral GE and non-GE canola populations in North Dakota, USA, beginning 4 June and continuing through 23 July 2010. Field crews established east-west transects on major roads throughout the state. A 1×50 m quadrat was established every 8.05 km (5 miles) of roadway on one or both sides of the road, where traffic permitted, in which all identifiable *B. napus* plants were counted. We drove a total of 5600 km and sampled 63.1 km of roadside habitats (1.1% of the

distance driven). Sampling was conducted early in the summer prior to the onset of flowering of cultivated canola. When canola was present at a sampling site, one randomly selected plant was collected, photographed and archived as a voucher specimen. Leaf fragments from voucher specimens were tested for the presence of CP4 EPSPS protein (confers tolerance to glyphosate herbicide) and PAT protein (confers tolerance to glufosinate herbicide) with TraitChek™ immunological lateral flow test strips (Strategic Diagnostics, Inc., Newark, DE). Previous studies have demonstrated the utility of the lateral flow strips in detecting the expression of transgenes from field samples [8,22]. Test strips are not available for a third, non-GE resistance trait, resistance to Clearfield™ herbicide, which comprises approximately 10% of the canola grown in the region (R Beneda, pers comm). At random intervals, single plants were tested with multiple test strips to assure that test results were repeatable and reliable. No failures were detected during the course of the study. To determine if populations of escaped canola are composed of multiple genotypes, multiple plants were sampled and tested for the presence of CP4 EPSPS or PAT proteins at 9 randomly selected, large canola populations Test strips and plant voucher specimens are archived at the University of Arkansas. GPS locations and transgene state values for each collected plant are available in Table S1.

Results

The escape of GE *B. napus* in North Dakota is extensive (Fig. 1). *Brassica napus* was present at 45% (288/634) of the road survey sampling sites. Of those, 80% (231/288) expressed at least one transgene: 41% (117/288) were positive for only CP4 EPSPS (glyphosate resistance); 39% (112/288) were positive for only PAT (glufosinate resistance); and 0.7% (2/288) expressed both forms of herbicide resistance, a phenotype not produced by seed companies (Table 1). Densities of *B. napus* plants at collection sites ranged from 0 to 30 plants m^{-2} with an average of 0.3 plants m^{-2}. Among the archived specimens, 86.8% were sexually mature varying in developmental stage from flower bud to mature fruit with seeds. At the time of roadside sampling, in-field canola was non-flowering having matured to the 4-leaf to pre-bolting stage (JPL pers. obs.). This striking difference in flowering phenology suggests that flowering canola in roadside habitats may have originated from the previous generation's seed bank rather than from seed spill during the current growing season.

Populations of transgenic canola were denser along major transport routes, at construction sites and in regions of intense canola cultivation (Fig. 1). At a finer scale, feral populations appeared denser at junctions between major roadways, access points to crop fields and bridges, and intersections of roadways with railway crossings. At these sites, seed spill during transport is a likely mechanism for the escape of transgenic canola. Nonetheless, feral *B. napus* plants were occasionally found at remote locations far from canola production, transportation, or processing facilities. Populations were also observed at roadsides that had recently been mowed or treated with herbicide. Although our sampling protocol stipulated that a single plant be tested at each collection site, multiple sampling of additional plants revealed a mix of both herbicide resistant phenotypes, or a mix of herbicide resistant and vulnerable phenotypes in all randomly-tested large populations (Table S1).

Discussion

To date there have been relatively few reports of the escape from cultivation of genetically engineered varieties leading some researchers to discount the environmental risks of biotech crops. Concurrently, public demonstrations have led to a consumer backlash against genetically engineered foods. A first step toward understanding the environmental impact of biotech crops is to identify the incidence and extent of their escape from cultivation.

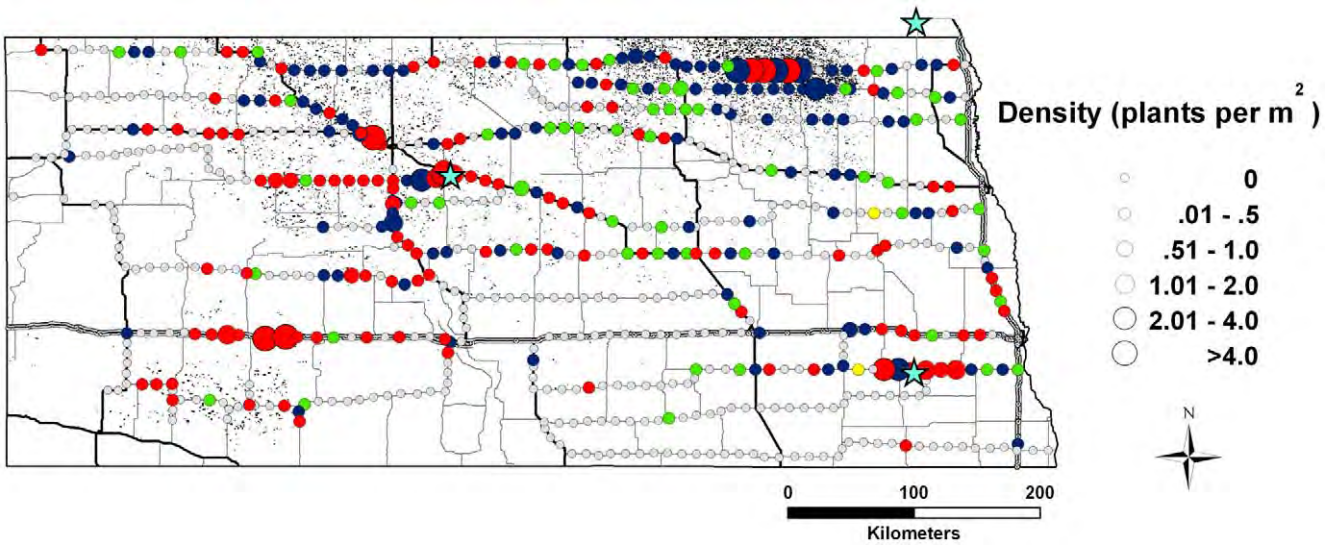

Figure 1. Distribution and density of feral canola populations in North Dakota road surveys (2010). Circles indicate locations of sampling sites; diameter of circle indicates plant density; gray circles indicate no canola present. The presence of genetically engineered protein in the vouchered specimen is shown by color: red – glyphosate resistance; blue – glufosinate resistance; yellow – dual resistance traits; green – non-transgenic. Canola fields are indicated by stippling based on 2009 USDA National Agricultural Statistics Service report (http://www.nass.usda.gov/Statistics_by_Subject/index.php?sector=CROPS). Stars show the locations of oilseed processing plants (3). Solid lines illustrate interstate, state and county highways.
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Table 1. Distribution of transgenic and non-transgenic canola in North Dakota transects.

	# of sites	Percent
Total transects	634	
Canola present	288	0.454
Transgenic	231	0.802
Liberty Link+	112	0.389
Roundup Ready+	117	0.406
LL+ and RR+	2	0.007
Non-Transgenic		
Null	57	0.198

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We conducted this study to document feral populations of genetically engineered canola and to evaluate potential mechanisms of persistence outside of crop fields.

The escape of canola from cultivation is not particularly surprising. *Brassica napus* is thought to have been domesticated very recently, in the last 300–400 years [23]. As a consequence, "wild" traits, such as seed shattering and partial seed dormancy, are still expressed in commercial canola and may contribute to escape from cultivation. For example, up to 30% of a seed crop may be lost each year by shattering during harvest [24] and canola seeds may remain dormant for up to three years [25]. The combined effects of seed loss on harvest and seed dormancy rapidly stock the soil seed bank, which can lead to frequent re-seeding of marginal soils [17].

Surprising from our study is the widespread distribution of feral canola outside of cultivated areas both near and far from cultivated fields over much of North Dakota and the likely persistence of these populations beyond single years. Additionally, these populations occur both in habitats with selection pressure (e.g., roadsides sprayed with glyphosate) and also in habitats without obvious selection pressure. Although canola cultivation in North Dakota occurs primarily in the northeastern counties, we identified transgenic canola populations in parts of North Dakota with little or no known canola production. Our results suggest a number of routes by which canola plants may be introduced to the wild. Feral canola populations were found in high densities along major trucking routes but not smaller tributaries suggesting that feral canola populations are established by seed spill. Similar results have been reported in studies of feral canola in Canada [17,18]. The mixture of phenotypes that we found in 9 large populations, further suggests that multiple seed spills or dispersal events can occur at a given location. In addition, we identified large, continuous populations of feral transgenic canola (population IDs 215–216) growing on fill dirt at highway construction zones that clearly did not result from seed shatter or seed spill (JPL pers. obs.). We suggest that canola may colonize repositories of fill dirt and rapidly establish a soil seed bank. The movement of contaminated fill dirt to remote construction sites provides an additional mechanism for the dispersal of transgenic canola far beyond field margins.

Movement by transport is likely to explain the current distribution of feral canola populations in North Dakota, but re-seeding by fertile plants further contributes to population persistence. Our evidence that these populations persist outside of cultivation includes the striking difference in flowering phenology between feral and commercial populations. Flowering times differed by approximately four weeks, indicating that field and feral populations originated from different sources. Further evidence for persistence is found in our statewide collections of fertile plants with viable seeds. Metapopulation dynamics by which feral populations are fed by seed transport but supplemented by *in situ* seed production are likely at play here as described by [18] for feral canola populations in Canada.

The occurrence of novel resistance phenotypes may provide additional evidence that these populations can persist outside of cultivation. When transgenic resistance genotypes grow in sympatry, varieties may hybridize to create novel combinations of traits, as we found at two locations. Because resistance to multiple herbicides has not been commercially developed in canola, the discovery of "stacked" traits in feral canola plants is evidence that biotech varieties have hybridized. Hybridization could possibly have occurred by pollen flow between fields of transgenic canola varieties, followed by seed spill along roadsides. Alternatively, hybridization could have occurred by pollen movement among resistant phenotypes within roadside populations, because feral populations were frequently found to include multiple phenotypes, or by flow of transgenic pollen from other feral populations or crop fields. By whatever mechanism, hybridization among genetically engineered varieties is not uncommon. Although we sampled a relatively small number of plants (N = 288) from a small percentage of the total potential habitat along roadways in North Dakota (1.1%), we nonetheless identified two individuals expressing novel stacked traits (0.7%). Furthermore, the incidence of crop-crop hybridization is undersampled in this survey because test strips for a third commercial form of herbicide resistant canola, Clearfield™, are not available.

These results support the hypothesis that roadside populations of canola in the U.S. are likely persistent from year to year, are capable of hybridizing to produce novel genotypes, and that escaped populations can contribute to the spread of transgenes outside of cultivation. Reports in Canada of feral populations of GE canola emerged soon after its commercial release there. Confirmation of GE pollen and crop movement among fields in Australia, U.K., Germany and France and Japan followed shortly thereafter. Ours is the first report of feral canola in the U.S. more than a decade after its commercial release. This delay raises questions of whether adequate oversight and monitoring protocols are in place in the U.S. to track the environmental impact of biotech products. At issue is the need to re-evaluate previous assumptions about crop systems: that crop genotypes outside of agriculture are not competitive; that protocols designed to reduce or prevent escape and proliferation of feral transgenic crops are effective; and that current tracking and monitoring of GE organisms are sufficient. Emerging pressures on agricultural systems by the accelerating growth of human populations argues that we take full advantage of the tools that biotechnology and conventional varietal development make available. It is essential that researchers, regulatory agencies and industry cooperate to ensure the continued security of food systems worldwide. The challenges of feeding a burgeoning global population in the face of limited and eroding natural resources requires substantial investments by all stakeholders. We must safely engage all tools available to us to advance food, fuel and fiber alternatives as modern agriculture rises to the challenges of the next decades.

Supporting Information

Table S1 Supplemental table of all collected *B. napus* populations.
(DOCX)

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Author Contributions

Conceived and designed the experiments: MGS AAR JPL EHL CLS. Performed the experiments: MGS AAR JPL SET CLS. Analyzed the data: MGS CAB EHL JPL CLS. Contributed reagents/materials/analysis tools: EHL PKV CLS. Wrote the paper: MGS JPL CAB EHL CLS. Publication costs: PKV CLS.

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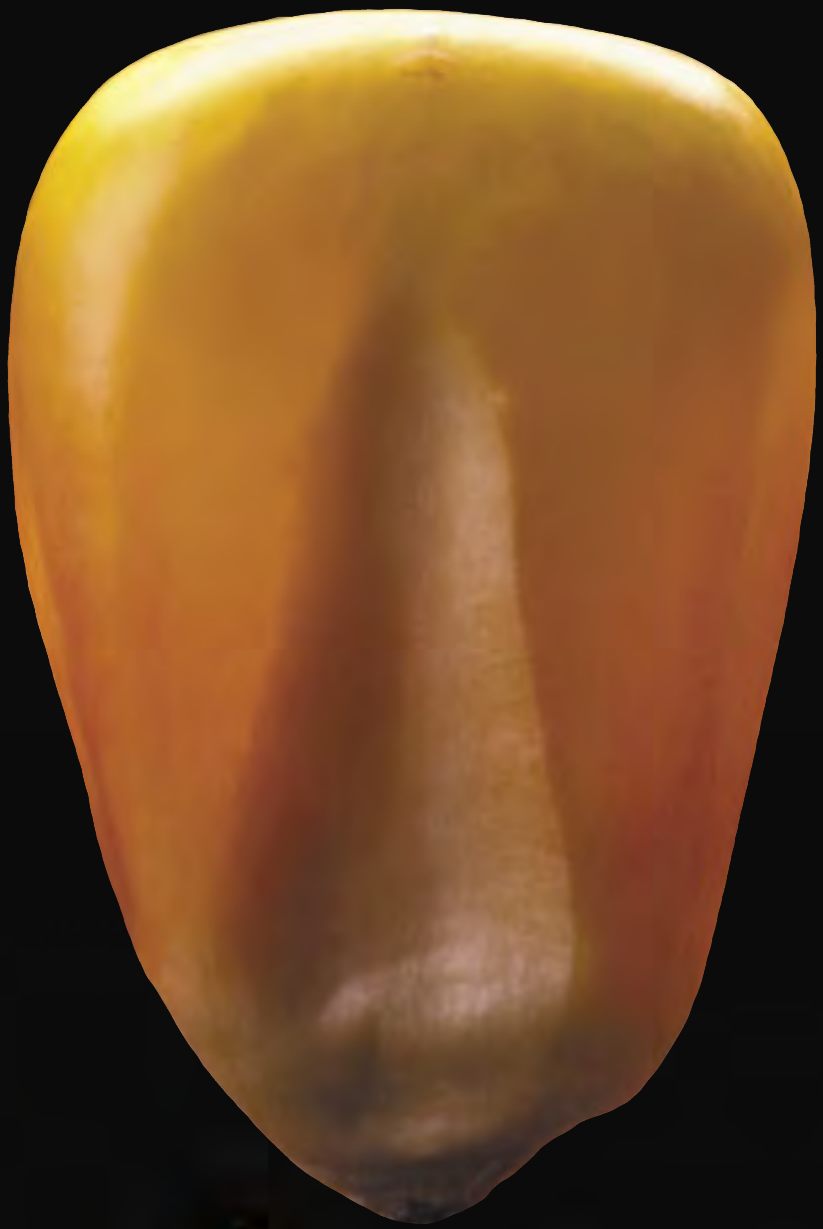
Gone to Seed

Transgenic Contaminants in the Traditional Seed Supply



Union of Concerned Scientists
Citizens and Scientists for Environmental Solutions

Gone to Seed

Transgenic Contaminants in the Traditional Seed Supply

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Union of Concerned Scientists
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The Union of Concerned Scientists is a nonprofit partnership of scientists and citizens combining rigorous scientific analysis, innovative policy development, and effective citizen advocacy to achieve practical environmental solutions.

The goal of the Union of Concerned Scientists Food and Environment Program is to create a food system that encourages innovative and environmentally sustainable ways to produce high-quality, safe, and affordable food, while ensuring that citizens have a voice in how their food is grown.

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Contents

Figures and Tables

FIGURES

TABLES

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The Union of Concerned Scientists is solely responsible for the contents of this report.

Executive Summary

Nothing is more fundamental to agriculture and our food supply than seeds. Whether eaten directly or processed through animals, seeds are the ultimate source of human nutrition. The variety, abundance, and safety of foods are all dependent on the availability and quality of seeds.

The prowess of genetic engineers notwithstanding, seeds cannot be made from scratch. They must be harvested, saved, and shepherded from generation to generation by knowledgeable, engaged individuals. The value to the food supply of the seeds entrusted to our generation cannot be overstated.

In this report, the Union of Concerned Scientists (UCS) examines a new phenomenon that may threaten the quality of the seed supply: the contamination of traditional seeds by DNA sequences derived from genetically engineered crop varieties. These varieties are produced by molecular techniques—variously known as genetic engineering, genetic modification, or transgenic techniques—that allow scientists to move novel traits into plants from distantly related organisms such as animals and bacteria.

The number of transgenes that might potentially contaminate the seed supply is large. Although most commercial transgenic varieties of corn, cotton, soybeans, and canola contain only two traits (herbicide and insect resistance), hundreds of other novel genes have been engineered into crops that have been field tested but have not been, and may never be, commercialized.

Most of the transgenes used by genetic engineers are new to foods and some are not intended for use in foods at all. For these and other reasons, concerns have arisen about the possibility that transgenes introduced into crop varieties through genetic engineering might unintentionally contaminate the seed supply for traditional, or non-genetically engineered, varieties of crops.

The research covered in this report addresses that possibility with a small pilot study of seeds of traditional varieties of three major food crops: corn, soybeans, and canola. The study found that the seeds of traditional varieties bought from the same retailers used by U.S. farmers are pervasively contaminated with low levels of DNA sequences originating in genetically engineered varieties of those crops.

Our conclusion:

Seeds of traditional varieties of corn, soybeans, and canola are pervasively contaminated with low levels of DNA sequences derived from transgenic varieties.

This conclusion is based on tests conducted by two respected commercial laboratories using duplicate samples of seeds of six traditional varieties each of corn, soybeans, and canola. One laboratory detected transgenically derived DNA in 50 percent of the corn, 50 percent of the soybean, and 100 percent of the traditional canola varieties tested. The other laboratory detected transgenically derived DNA in 83 percent of the traditional varieties of each of the three crops. The most

conservative expression of the combined results is that transgenically derived DNA was detected in 50 percent of the corn, 50 percent of the soybean, and 83 percent of the canola varieties tested.

Other than suggesting that the levels are low, the pilot study is too limited to support quantitative estimates of overall contamination levels in seeds of traditional crop varieties. The data available lead us to expect levels of contaminated seed roughly in the range of 0.05 to 1 percent, but larger studies are needed to determine contamination levels with any degree of precision.

In the interim, we are concerned that the significance of low-level contamination might be too quickly dismissed. Contamination levels in the 0.05 to 1 percent range would represent huge absolute amounts of seed. To illustrate, we calculated the tonnage of transgenically contaminated corn seeds that would have been planted in fields of traditional corn varieties if the seed supply were contaminated at a one percent rate. Our calculations, based on U.S. Department of Agriculture (USDA) data on corn acres planted with traditional varieties in 2002, suggest a total of 6,250 tons of transgenically derived seeds—an amount that would fill 240 large tractor-trailer trucks.

Most of the specific DNA sequences for which the laboratories tested are found in popular transgenic crop varieties currently allowed on the U.S. market. Although the study sheds little light on how the seed contamination occurred, there is no reason to believe that the transgenes detected in this study are the only ones moving into the traditional seed supply.

Instead, it seems likely that the contamination is a symptom of generally porous seed production and distribution systems. Until we know otherwise, it seems minimally prudent to assume that novel genes originating in less popular transgenic varieties, as well as the hundreds of engineered varieties that have been field tested in the United States, could potentially contaminate the seed supply of food and feed crops.

IMPLICATIONS

The recognition that the seed supply is open to contamination by low levels of a wide variety of genetically engineered sequences has broad implications. In general terms, seed contamination is important for two reasons. First, seeds reproduce and carry genes into future generations. Every season of seed production offers new opportunities for the introduction of new genes. In the case of genetic engineering, transgenic sequences that enter the seed supply for traditional crop varieties will be perpetuated and will accumulate over time in plants where they are not expected and could be difficult to control.

Second, seeds are the wellspring of our food system, the base on which we improve crops and the source to which we return when crops fail. Seeds will be our only recourse if the prevailing belief in the safety of genetic engineering proves wrong. Heedlessly allowing the contamination of traditional plant varieties with genetically engineered sequences amounts to a huge wager on our ability to understand a complicated technology that manipulates life at the most elemental level. Unless some part of our seed supply is preserved free of genetically engineered sequences, our ability to change course if genetic engineering goes awry will be severely hampered.

Seed contamination by transgenically derived sequences also has implications in a number of other regulatory and policy contexts. Pharm crops, trade, and organic food production are discussed briefly in this summary, but our report also addresses implications for food safety, the environment, intellectual property, the food system, and the agriculture of developing countries.

Pharmaceutical and industrial crops receive special attention in this report because the trans-

genic products they make—drugs, vaccines and industrial chemicals—would raise immediate alarms if they contaminated the food supply, and seed contamination is the back door to the food supply. The realization that seeds for food crops are vulnerable to contamination with pharm and industrial transgenes and that, in fact, some seeds may already have been contaminated is alarming. The report urges prompt action to protect seed production from these sources of contamination.

On the trade front, U.S. grain and oilseed exporters face enormous challenges in a global marketplace bristling with regulatory regimes that apply to genetically engineered crops. U.S. companies need to assure export customers that grain and oilseed shipments do not contain unapproved transgenes and transgenic crop varieties. While gene flow and physical commingling during production and transport probably account for most of the unapproved transgenes and transgenic seed varieties present in exported grain and oilseed, traditional crop varieties carrying transgenically derived sequences may also contribute to the problem. Contamination of the seeds of traditional plant varieties also makes it difficult to supply commodity products free of genetically engineered sequences to those customers who want them.

Transgenic contamination of traditional seed varieties poses a special threat to the future of organic agriculture, an increasingly important sector of U.S. agriculture. To meet both consumer demand and federal standards that forbid the use of genetically engineered crops and inputs, organic growers strive to produce crops that are free of transgenically derived DNA. If, through no fault of their own, they are unable to supply such products, they potentially face eroding markets. The ease with which the traditional seed supply can be contaminated with transgenically derived DNA unfairly frustrates organics farmers seeking to deliver high-quality products.

RECOMMENDATIONS

UCS hopes that, as a result of this report, the seed and food industries, the scientific community, and the federal government will begin to acknowledge and confront the issues raised by the contamination of the traditional seed supply with sequences originating in genetically engineered crops. While not entirely reversible, this contamination can be substantially reduced. With sufficient attention and will, it is possible to look forward to sources of seeds that are free of genetically engineered sequences. The first step, however, is acknowledging and understanding the problem.

More specifically, UCS recommends the following actions:

1. The USDA should sponsor a full-scale investigation of the extent, causes, and impacts of contamination of the traditional seed supply by transgenically derived DNA sequences.

2. The USDA, the Food and Drug Administration, the Environmental Protection Agency, and appropriate coordinating elements of the federal government should amend the regulations for transgenic pharm and industrial crops to ensure that the seed supply for food and feed crops is not contaminated at any level with drugs, vaccines, plastics, or related substances.

3. The USDA should establish a reservoir of seeds for non-engineered varieties of major food and feed crops free of transgenically derived sequences.

4. The USDA and land-grant (agricultural) universities should reinvigorate the public plant breeding establishment to help ensure a supply of pure seed of traditional crop varieties.

5. The Association of Official Seed Certifying Agencies should establish a national standard

for breeder and foundation seed of traditional crop varieties: no detectable level of contamination by transgenes and associated sequences originating in genetically engineered crops.

6. The USDA, the organic agriculture community, land-grant universities, and plant breeders should develop new policies and programs to provide organic agriculture with pure seeds of traditional crop varieties.

7. The USDA, the organic and biotechnology industries, and national growers' associations, among others, should sponsor a series of meetings to begin addressing how those sectors of U.S. agriculture that have adopted transgenic crops and those threatened by contamination with transgenically derived DNA sequences from those crops can coexist.

8. Private seed companies in the United States should periodically test their seed stocks, especially breeder and foundation seed and parental inbred lines, for the presence of transgenically derived DNA sequences. They should then make public the extent to which the seeds of the traditional varieties they market are free of transgenically derived contaminants.

Chapter 1

Introduction

This report describes the results of a pilot study designed to address the extent to which genetic elements introduced into the crop gene pool via genetic engineering are now present in crop varieties with no history of genetic engineering. The results suggest that seeds representing a wide array of corn, soybean, and canola varieties currently on the market commonly contain identifiable genetic material originating from transgenic crop varieties.

The varieties collected for analysis in this study were produced by traditional, field-based plant breeding techniques. These techniques rely on identifying and mating parent plants that possess promising traits and repeatedly selecting for superior performance among their offspring. Seeds for offspring that do well in performance trials are then increased prior to sale as a commercial crop variety. Traditional plant breeding, a potent technology often taken for granted, is largely responsible for the tremendous gains in productivity of global agriculture during the twentieth century. (See Figure 1-1, p. 8, and Appendix A for more information on variety development and seed production.)

The sources of the novel genetic elements that now appear to contaminate the seed supply of traditionally bred crop varieties are varieties created by newer molecular-level laboratory techniques. These techniques, collectively known as genetic engineering, allow scientists to insert and express genetic material originating in organisms unrelated to the crops in question.

Unlike traditional breeding methods that rely on mating between male and female parents to generate new or improved traits, laboratory-based techniques can move genetic material directly into plants from organisms as distantly related as bacteria or animals. These techniques are also referred to as genetically modified or transgenic. The organisms produced by these techniques are referred to as genetically engineered organisms, genetically modified organisms (GMOs), and transgenics.

In this report, we will refer to crop varieties with no history of genetic engineering as traditional varieties and the seeds for those varieties as traditional seeds or the traditional seed supply. Crop varieties produced via genetic engineering techniques are described as transgenic, although we recognize that field-based techniques used to develop traditional varieties are also used in the production of commercial transgenic varieties. Transgenic seeds or the transgenic seed supply refers to seeds used to grow transgenic crop varieties.

The DNA sequences introduced into plants during the genetic engineering process are referred to as transgenically derived or transgenic sequences, and novel genes transferred to crops using genetic engineering techniques are referred to as transgenes. Biochemical techniques that make it possible to identify specific DNA sequences, even at very low levels, were critical to conducting this study.

GENETIC ENGINEERING IN AGRICULTURE

Genetic engineering has been a controversial technology from the beginning, especially in Europe and other countries outside the United

States. Concerns about the use of the technology in agriculture have focused on a tangle of issues ranging from concerns about food and feed safety to environmental risk and corporate control of the food system.

In theory, genetic engineering can modify plants to produce a wide range of new traits. Yet most engineered varieties commercially planted in the United States and around the world have been modified to express only two narrow categories of traits: resistance to a particular herbicide (thus permitting the use of that herbicide) or the expression of a pesticidal toxin derived from the soil bacterium *Bacillus thuringiensis* (Bt). These are referred to as herbicide-resistant and insect-resistant (or Bt) varieties, respectively.[1]

Bt and herbicide-resistant versions of major crops were first planted on a large scale in 1996 and have been widely adopted in the United States during the last few years.[2] In 2002, for example, about three-fourths of U.S. soybean acres, one-third of U.S. corn acres,[3] and nearly 70 percent of North Dakota's canola acres[4] were planted with engineered varieties. (North Dakota accounts for 89 percent of U.S. canola production.[5]) Traditional, or non-engineered, crop varieties nevertheless remain popular as well, and U.S. farmers continue to plant them in large quantities.[6]

This study is the first systematic attempt to examine a part of the contamination issue that so far has received little attention: the extent to which the traditional seed supply for commodity crops has become contaminated with genetic sequences originating from transgenic varieties.

In addition to the handful of transgenes present in commercial varieties of herbicide-resistant and Bt crops, hundreds of other transgenes have been engineered into crops. These varieties, though not yet commercialized, have been field tested in the open environment. Appendix B of this report contains a list of transgenes and transgenic traits taken from a database of U.S. Department of Agriculture (USDA) records of field tests of corn, soybeans, and canola over the past 16 years.[7]

Because transgenic and traditional varieties of major crops are both planted widely and moved

1 Union of Concerned Scientists (UCS). 2002. Genetically Engineered Foods Allowed on the Market. Cambridge, MA: UCS. On the UCS website at *http://www.ucsusa.org/food_and_environment/biotechnology/page.cfm?pageid=337*, accessed on August 13, 2003. Several herbicide-resistant and Bt varieties are on the market in the United States, including canola, corn, and soybeans resistant to glufosinate and glyphosate herbicides; cotton resistant to glyphosate and bromoxynil herbicides; and Bt corn and Bt cotton.

2 For information on the growth in acreage of genetically engineered crops in the United States and elsewhere, see International Service for the Acquisition of Agri-biotech Applications (ISAAA) Briefs on the ISAAA website at *http://www.isaaa.org*.

3 U.S. Department of Agriculture, National Agricultural Statistics Service (USDA NASS). 2003. Prospective Plantings. March 23, pp. 20, 21on the USDA NASS website at *http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bbp/pspl0303.pdf*, accessed on August 15, 2003.

4 Berglund, D.R. 2003. Personal communication, August 15. D.R. Berglund is a professor and extension agronomist at North Dakota State University. According to Dr. Berglund, approximately 900,000 of North Dakota's 1,300,000 acres of canola were planted with engineered varieties in 2002.

5 USDA NASS. 2003. Crop Production: 2002 Summary. Publication CrPr2-1(03). p. 31. On the USDA NASS website at *http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bban/cropan03.pdf*, accessed on November 25, 2003.

6 Traditional crop varieties remain popular for a number of reasons, including the large international markets for such varieties, the relatively high price of seeds for engineered varieties, and personal preferences.

7 Information Systems for Biotechnology (ISB). 2003. Field Test Releases in the U.S. Blacksburg, VA: Virginia Polytechnic Institute and State University. On the ISB website at *http://www.isb.vt.edu/cfdocs/fieldtests1.cfm*, accessed on December 15, 2003.

together through the U.S. grain distribution system, there are many activities that can mix the two kinds of crops. Most of the commercial bulk oilseeds and grains in the United States, for instance, are now a mixture of engineered and non-engineered seeds. As discussed below, this high degree of commingling has made it difficult for the United States to segregate and deliver a non-genetically engineered product for customers who demand it.

THE CURRENT SITUATION

This study is the first systematic attempt to examine a part of the contamination issue that so far has received little attention: the extent to which the *traditional seed supply* for commodity crops has become contaminated with genetic sequences originating from transgenic varieties.

We use the term "contamination" here to refer to seeds or genetic sequences that are unwanted in a particular place for one reason or another. Corn, for example, is unwanted in shipments of soybeans and in such shipments is properly called a contaminant. The term has no negative connotation other than the sense that a particular entity is for some reason unwanted or inappropriate where it is found.

"Adventitious presence," another term sometimes heard in this context, connotes a lack of intention in allowing commingling to occur. Adventitious presence in our view is a broader term than contamination. Contamination refers to those situations where genes or traits are not only unintended (or adventitious) but also for some reason unwanted.

Both commercial and legal considerations make the presence of transgenically derived sequences in agricultural products problematic. Many transgenic varieties of crops in use in the United States have not been approved in other countries and their presence in imports is unlawful. In addition, many customers for U.S. exports —particularly those looking to purchase organic food or non-organic specialty products—are exhibiting a strong preference for non-genetically engineered grains and oilseeds free of some or all transgenic varieties.

Seeds in commodity agriculture

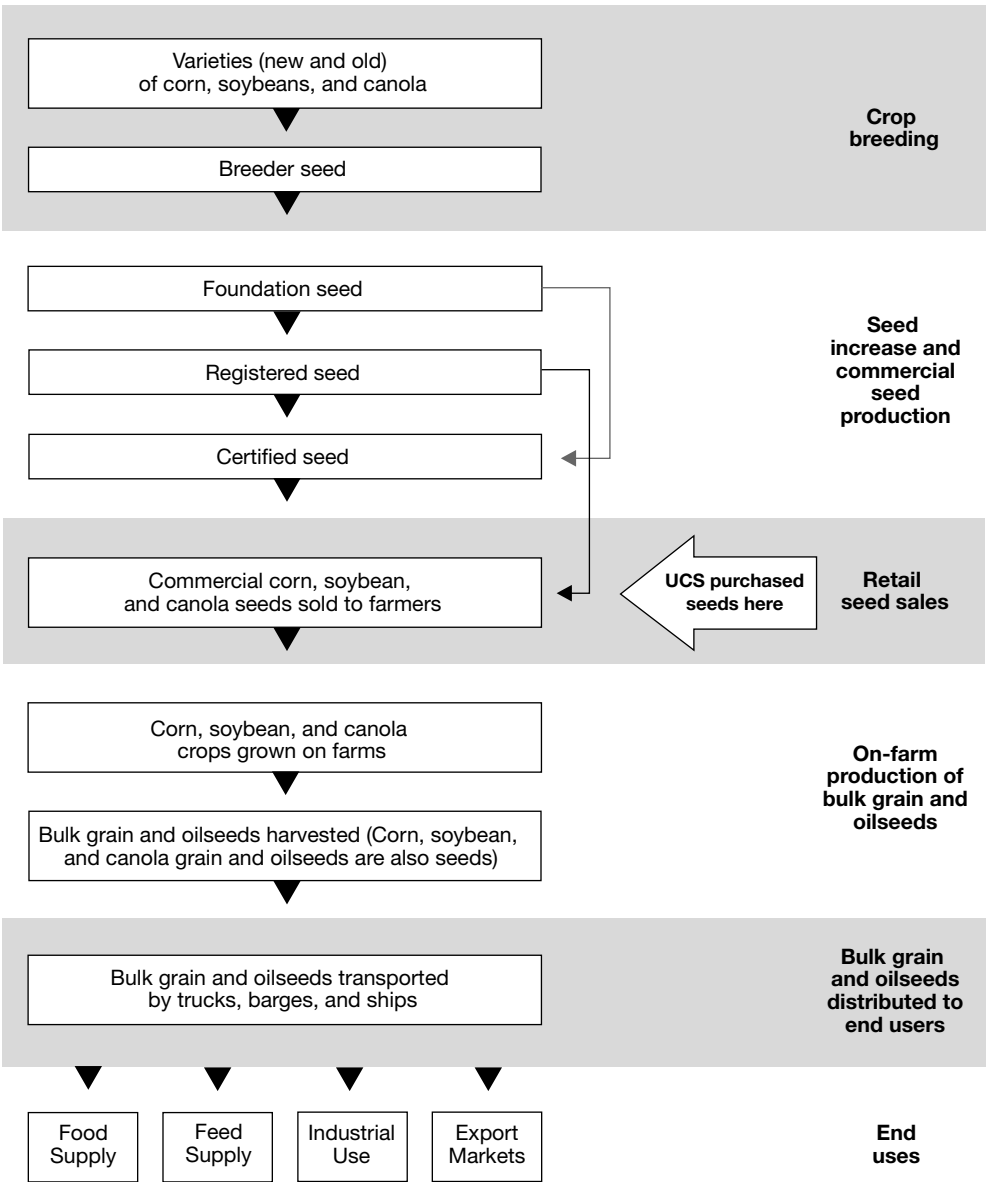
Each season, farmers plant seeds of commodity crops such as corn, canola, and soybeans to produce a crop that will be harvested and sold as bulk grain and oilseed. Figure 1-1 (p. 8) illustrates how seeds of corn, soybean, and canola varieties move through the agricultural commodity system. For a more detailed account of crop variety development and seed production, see Appendix A.

Plant breeders are constantly producing new varieties of corn, soybeans, and canola. Every year a set of varieties (old and new) is selected for commercial development and a process called seed increase is set in motion to generate sufficient quantities of seeds to be offered for sale to growers. Seed increase usually requires several rounds of planting and harvesting to meet commercial demands.

For economic reasons, seeds are grown under progressively less stringent containment conditions, which correspond to four classes of seed purity. Breeder seed, controlled by the plant breeding institution, is the purest class of seed, followed by foundation, registered, and certified seed (the least pure class). Private certifying agencies set crop-specific purity standards for each seed class. Examples of corn, soybean, and canola seed standards can be found in Appendix A.

Farmers can obtain commercial seed through retail seed stores, the Internet, and catalogs. Seeds purchased by growers are planted, the plants are tended during the growing season, and seeds are harvested and sold as bulk grain and oilseed products. Eventually these products make their way to end users for a variety of purposes including feed, food, and industrial uses. Substantial quantities of U.S. grain and oilseeds are exported to other countries. Although rarely done in the case of corn, farmers may also retain soybean or canola seed from their harvest to plant the following year.

Figure 1-1 **Seeds in Commodity Agriculture: How Seeds of Corn, Soybean, and Canola Varieties Move from Plant Breeders to End Users**



Many of these customers are rejecting grains and oilseeds containing detectable levels of transgenic varieties regardless of whether the levels or kinds of transgenic varieties render the product technically illegal. In both legal and commercial contexts, the unwanted presence of genetically modified grains or oilseeds, and sequences deriving from them, are therefore properly considered contaminants.

SEED VS. BULK CROP CONTAMINATION

As mentioned above, seed contamination, the focus of this report, is only one source of the contamination that bedevils exporters of non-engineered bulk grain and oilseeds.

Most contamination is attributable to events that occur *after the engineered and non-engineered varieties of seed are planted* (Figure 1-1). There are two types of mixing events that occur after

planting: physical mixing, such as commingling in grain elevators; and outcrossing, the movement of genes via pollen into neighboring fields of sexually compatible crops. Since both of these phenomena are difficult to control under the current systems of grain and oilseed production, transport, and storage, mixing would occur even if the seeds farmers planted were absolutely free of transgenically derived sequences. While starting with seed contaminated with transgenically derived sequences exacerbates these problems, pure seed would not alleviate them.

EARLY WARNINGS

When transgenic varieties were first allowed on the market in the United States, little attention was paid to the idea that widespread adoption of transgenic crops could lead to seed contamination of traditional varieties. In retrospect, this seems surprising. Breeders working with genetically engineered varieties continued to use the same seed purity standards that applied to traditional varieties. Those standards vary from crop to crop but allow, in the case of soybeans, for example, up to 0.6 percent of the seeds to come from other kinds of crops such as corn and up to 0.5 percent from other varieties of soybeans (Appendix A). Application of these standards made it almost inevitable that substantial cross contamination would follow the widespread adoption of genetically engineered crop varieties.

A number of factors—among them, the growing global controversy over biotechnology crops, the increasing popularity of organic foods, and regulatory regimes that vary from country to country—have led to demands for crops of far greater purity than the seed production system was geared to deliver. But awareness of this situation emerged slowly. Plant breeders, growers, and others in the agricultural establishment seemed to proceed on the assumption that even as the adoption rates of genetically engineered varieties increased, those who wanted to purchase seed free of transgenic components would be able to continue doing so.

A number of instances of seed contamination over the last seven years have called that optimistic assumption into question.

StarLink-contaminated hybrid corn seed

StarLink was an engineered corn variety approved by the U.S. government in 1997 for use in animal feed but not in human food. In September 2000, after newspapers reported that StarLink corn was showing up in consumer products, the government undertook comprehensive testing of corn-derived foods in the U.S. food supply.[8] Although planted on only 350,000 of the 80 million total U.S. corn acres (about 0.4 percent) in its most popular year,[9] genetic sequences from StarLink corn varieties were eventually detected in numerous consumer products distributed throughout the U.S. food supply and in exported corn.

By 2001, StarLink also contaminated the U.S. corn seed supply. Fearing recurrent introduction of the illegal contaminant into food via the seed supply, the USDA instituted a program to buy up corn seed that tested positive for StarLink. In June 2001, the department announced that it had already purchased $13 million worth of StarLink-contaminated seed from 63 companies and was

8 Taylor, M.R. and J.S. Tick. 2003. Post Market Oversight of Biotech Foods: Is the System Prepared? Washington, DC: Pew Initiative on Food and Biotechnology, pp. 90-105.

9 Keller, D. and D. Miller. 2000. Biotech's black eye. *Progressive Farmer* (December), p. 24; USDA NASS. No date. U.S. corn acres. On the USDA NASS website at *http://www.usda.gov/nass/aggraphs/cornac.htm*, accessed on December 2, 2003.

Almost half the organic growers surveyed recently felt that contaminated seeds represented the greatest source of contamination from engineered varieties.

considering additional expenditures of up to $5 million.[10]

Despite concerted effort, it has proved surprisingly difficult to purge the U.S. grain system of the contaminant. As recently as December 2003, StarLink was still being reported in domestic grain.[11] Part of the explanation may be that the seed supply for corn is still contaminated. It may be that inbred lines remain contaminated with StarLink genetic sequences and every time these inbreds are used to produce hybrid corn seed, the StarLink sequences are reintroduced into the seed supply. (See Appendix A for details on hybrid corn seed production.)

Contaminated foundation soybean seed

In 2002, the head of North Dakota State University's Foundation Seedstocks Program acknowledged that the program's foundation seed for non-engineered natto soybeans—the basic stock from which seeds are grown to sell to farmers—contained sequences from engineered soybeans.[12] (Natto soybeans are grown for premium food-grade products.) Three other foundation soybean seed programs—in Virginia, Missouri, and Michigan—have also recently reported genetic engineering contamination problems.[13]

Contaminated canola seed

In 1997, Monsanto, a leading biotechnology company, recalled 60,000 bags of seeds of one of its transgenic canola varieties in Canada because they were contaminated with seeds of another transgenic canola variety (RT-200), which had not been approved for marketing in that country.[14] Four years later, Monsanto detected the RT-200 contaminant again in seeds of commercial transgenic canola varieties in Canada. Even though RT-200 varieties had gained approval in Canada by that time, Monsanto withdrew the contaminated seeds from the market because the contaminating varieties had not been approved in all countries to which Canadian canola would be exported.[15]

Monsanto admitted in 2002 that RT-200 seeds might also have been contaminating U.S. canola seed supplies since 1999. Even though the company has no plans to commercialize RT-200 in the United States, it sought approval of the

10 USDA. 2001. USDA purchases Cry9C affected corn seed from seed companies. Press release, June 15, 2001. On the USDA website at *http://www.usda.gov/news/releases/2001/06/0101.htm*, accessed on November 14, 2003.

11 Fabi, R. 2002. Global updates: Exporters say Japan finds StarLink in U.S. corn cargo. Reuters, December 28; Jacobs, P. 2003. Banished biotech corn not gone yet: traces raise health, other key issues. *San Jose Mercury News* (December 1). On the *Mercury News* website at *http://www.bayarea.com/mld/mercurynews/business/7386106.htm*, accessed on December 2, 2003.

12 Pates, M. 2002. Seed contamination raises control issues, posted November 12, 2002. On the *Grand Forks Herald* website at *http://www.grandforks.com*, accessed on January 7, 2003. The article identified Monsanto's Roundup Ready soybeans as the source of contamination.

13 *The Non-GMO Source*. 2003. Concerns increase over GMO contamination of foundation seed. Volume 3, Number 6, pp. 1-2, June.

14 Rance, L. 1997. Registration suspended: Genetic mixup prompts recall of Roundup Ready canola. *Manitoba Co-Operator* (April 24).

15 Monsanto. 2001. Press statement: Quest canola seed replacement offered, April 25. On the Monsanto website at *http://www.monsanto.com/monsanto/media/01/01apr25_quest.htm*, accessed on December 18, 2001.

variety in this country that year to minimize the disruption caused by its contamination of other canola varieties.[16]

In the spring of 2000, Advanta Seeds UK acknowledged that traditional canola varieties contaminated with an engineered variety (GT-73) had been sold to several European Union (EU) countries—where it had not been approved for sale—in 1999 and 2000.[17] In 2002, Scottish scientists discovered that transgenic canola plants being tested in field trials were contaminated with a transgene not approved for testing in the United Kingdom.[18]

Organic producers struggle to find non-engineered seed

Organic food and fiber is one of the fastest-growing sectors in U.S. agriculture. Not only do many consumers expect organic food to be free of genetically engineered material, but federal standards also forbid the use of genetically engineered varieties in the production of organic foods. Organic growers seeking to meet this standard are finding it increasingly difficult to obtain non-engineered seed. Almost half the organic growers surveyed recently felt that contaminated seeds (rather than post-planting pollen drift, for example) represented the greatest source of contamination from engineered varieties.[19] The difficulty in producing pure seed has led some organic seed companies to move their seed operations outside the United States.[20]

GOVERNMENT'S FAILURE TO RESPOND

While any one of these incidents might reflect an isolated example of seed contamination, taken together they reasonably suggest a more widespread phenomenon. The prospect of broad contamination of the seed supply raises important questions for food safety, international trade, organic agriculture, and the integrity of the seed system at the base of our global food supply.

The growing evidence of seed contamination should have prompted the U.S. government to determine the extent to which seeds marketed as non-engineered are currently contaminated with engineered sequences. Indeed, the Union of Concerned Scientists (UCS) and others in the public interest community have suggested the government undertake such a study. But it has not responded.

The growing evidence of seed contamination should have prompted the U.S. government to determine the extent to which seeds marketed as non-engineered are currently contaminated with engineered sequences.

16 Hesman, T. 2002. Monsanto says gene-altered food may be in U.S. food. *St. Louis Post-Dispatch* (Business, April 16); Kilman, S. and J. Carroll. 2002. Monsanto admits unapproved seed may be in crops. *Wall Street Journal* (April 15).

17 Brown, N. 2000. Statement of the United Kingdom Minister of Agriculture, Fisheries and Food in the House of Commons, May 18. On the United Kingdom Parliament website at *http://www.publications.parliament.uk/pa/cm199900/cmhansrd/vo000518/debtext/00518-09.htm*, accessed on June 18, 2003.

18 Kelbie, P. and M. Woolf. 2002. Ministers suspend GM crop-testing. *The Independent* (August 16). Obtained from the *biotech_activists@iatp.org* mailing list server August 16, 2002, where the source was listed as *The Independent* website at *http://news.independent.co.uk/uk/environment/story.jsp?story=324776*. Apparently, the contaminated seeds, provided by Aventis (a biotechnology company now owned by Bayer Crop Science), had been planted in more than 20 test plots over a three-year period in England and Scotland.

19 Organic Farming Research Foundation (OFRF). 2003. Preliminary results from OFRF's fourth national organic farmers' survey: Section 7—GMOs and organic. On the OFRF website at *http://www.ofrf.org/press/releases/pr.051403.gmosurvey.html*, accessed on June 19, 2003.

20 *The Non-GMO Source*. 2003. Organic seed company moves corn production to Argentina to avoid GMOs. Volume 3, Number 1, p. 3, January.

So, UCS decided to conduct a pilot study of its own to assess the extent of contamination in the U.S. traditional seed supply. These seeds, along with seeds for transgenic varieties, are available from seed retailers, by mail order, and over the Internet.

As described below, our study found low levels of transgenically derived sequences in most of the samples of non-engineered corn, canola, and soybean seeds that we tested. The samples were obtained from seed sold in a number of locations around the United States. Our results suggest that the U.S. supply of seed for traditional varieties of corn, soybeans, and canola is pervasively contaminated with low levels of genetic sequences originating in transgenic varieties.

IDENTITY-PRESERVATION SYSTEMS

The purity of seed is an issue of growing interest outside the arena of genetic engineering. New efforts are under way to create value-added markets for high-value crops, including some produced by genetic engineering. High-value crops exhibit desirable traits such as increased levels of important nutrients or the ability to produce a drug or industrial chemical. Today's commodity system, which minimizes transportation, cleaning, and handling costs in part by tolerating a relatively high degree of cross-contamination, cannot meet the need for segregated, pure supplies of these high-value crops.

Spurred by market demand, individuals and companies are taking on the challenge of developing new infrastructure and delivery systems for value-added products.[21] New "identity-preserved" systems create alternative pathways between seed suppliers, growers, and customers that avoid the current commodity system and its endemic sources of cross-contamination.[22]

The U.S. government is currently exploring ways to facilitate the marketing of identity-preserved products. For example, the USDA is considering ways to reconfigure the commodity grain system to make segregation more feasible.[23] Fundamental to the new systems devised to "preserve identity" is the ability to produce and preserve the purity of seed.

REPORT OUTLINE

Chapter 2 describes how we conducted our seed study and what we found. Our analysis suggests that the contamination of commercial seed stocks is pervasive and ongoing, and that the current regulatory regimes, which were not designed to prevent such contamination, are incapable of doing so. Because seed stocks are fundamental to agriculture and the food supply, seed contamination has potential implications in a number of arenas. It is time to understand and address these implications.

We have attempted to initiate a discussion of these issues in Chapter 3, where we consider the implications of contamination in nine contexts. The most urgent of these is what many in agriculture expect to be the next big wave of biotechnology applications: crops that produce pharmaceuticals and industrial chemicals. Other contexts include food safety, the environment,

21 It is important to note that these systems are designed to respond to commercial, not safety, considerations.

22 Strayer, D. 2002. *Identity-Preserved Systems: A Reference Handbook*. Boca Raton, FL: CRC Press; Sundstrom, F.J., J. Williams, A. Van Deynze, and K.J. Bradford. 2002. Identity Preservation of Agricultural Commodities. Agricultural Biotechnology in California Series, Publication 8077. Davis, CA: University of California, Davis. On the UC Davis website at *http://anrcatalog.ucdavis.edu*, accessed on May 30, 2003.

23 USDA, Grain Inspection, Packers and Stockyards Administration (GIPSA). 2000. Request for public comments on how USDA can best facilitate the marketing of grains, oilseeds, fruits, vegetables, and nuts in today's evolving marketplace. *Federal Register* 65:21272-21273 (November 30); USDA GIPSA. 2002. Facilitating the marketing of U.S. agricultural products with new testing and process verification services. *Federal Register* 67:50853-50854 (August 6).

trade, organic food production, intellectual property, the food system, agriculture of developing countries, and seed repository integrity.

In Chapter 4, we present our conclusions and recommendations for further research and new policies.

The main text of the report is followed by a glossary and two appendices. Appendix A provides an overview of plant breeding and seed production. Appendix B lists transgenes and transgenic traits engineered into corn, soybeans, and canola for field testing purposes since 1987.

Chapter 2

Methods and Results

UCS's pilot study looked at the contamination of the traditional seed supply in three major commodity crops: corn, soybeans, and canola. The seeds tested were selected from the pool of seeds marketed by major seed companies in 2002 to growers in key agricultural states. Selection procedures were developed to ensure that, to the degree possible given our limited resources, the seeds tested were representative of a large portion of the traditional seed supply for these crops.

This chapter describes the study, its results, and its limitations. Text boxes explain the basics of plant genetic engineering and designations used in the text and tables. A glossary is found at the end of the report.

METHODS

Choosing crops

In late 2001, there were 11 crops that had cleared the regulatory hurdles for marketing in the United States.[24] Among these, only four had engineered versions that had been widely planted: canola, corn, cotton, and soybeans. We eliminated cotton because it is not used primarily for food.

Choosing varieties

The next step was to decide, given limited resources, how to sample the available seeds. To sample as large a portion of the 2002 seed supply as possible, we selected from the pool of non-engineered varieties offered by major seed companies to growers in states that have significant acreage dedicated to the three crops.

For corn and soybeans, specifically, we selected varieties from among those recommended by major seed companies to growers in Iowa and Illinois, the states with the most acreage dedicated to those two crops.[25] From the websites of four major seed companies, we obtained lists of traditional varieties recommended for various counties or zip codes in Iowa and Illinois.[26] We chose to focus on one county in each state—Polk in Iowa and Wabash in Illinois. Seed companies recommended anywhere from 2 to 40 traditional seed varieties for those two counties (or zip codes within them), and we chose two from each of three companies. That gave us six varieties of corn and six of soybeans.

Where a company recommended more than two varieties for one of those locations, we

24 Union of Concerned Scientists (UCS). 2002. Genetically Engineered Foods Allowed on the Market. On the UCS website at *http://www.ucsusa.org/food_and_environment/biotechnology/page.cfm?pageid=337*, accessed on August 13, 2003. The 11 crops allowed on the market were canola, corn, cotton, flax, papaya, potato, radicchio, soybean, squash, sugar beet, and tomato.

25 According to USDA National Agricultural Statistics Service (USDA NASS) data, Iowa and Illinois planted more acres with corn and soybeans than any other states in the 2002 growing season (*http://www.usda.gov/nass/aggraphs/cornacm.htm* and *http://www.usda.gov/nass/aggraphs/soyacm.htm*, accessed on May 15, 2003).

26 Major seed companies maintain websites where farmers can find the varieties recommended for their area by entering either their county name or zip code. Between December 2001 and February 2002, we obtained lists of recommended varieties for the 2002 growing season in Illinois (Wabash County; zip code 62806) and Iowa (Polk County; zip code 50011) from the following seed companies' websites: Monsanto/Asgrow at *http://www.farmsource.com*, Syngenta at *http://www.nk-us.com*, DuPont/Pioneer at *http://www.pioneer.com*, and Dow/Mycogen at *http://www.mycogen.com*.

TABLE 2-1 **Traditional Varieties of Corn, Soybeans, and Canola Selected for This Study**

Crop	Company Producing Seeds of Traditional Varieties	Seeds of Traditional Varieties Purchased		
		Variety Designation Used in This Report	Company Variety Designation*	From a Seed Retailer in:
Corn	DuPont/Pioneer	1	36B08	Clarke County, VA
		2	34G13	Clarke County, VA
	Syngenta	3	N60-N2	Edwards County, IL
		4	V72-V7	Frederick County, VA
	Dow/Mycogen	5	5212	Frederick County, VA
		6	2A791	Frederick County, VA
Soybean	DuPont/Pioneer	7	94B53	Edwards County, IL
		8	93B82	Clarke County, VA
	Syngenta	9	S25-J5	Edwards County, IL
		10	S42-H1	Edwards County, IL
	Monsanto/Asgrow	11	A2869	Edwards County, IL
		12	A4922	Jefferson County, WV
Canola	Proseed	13	Topscore	Wells County, ND
		14	Canterra 1492	Wells County, ND
	Interstate	15	Hyola 330	Cass County, ND
		16	Hyola 401	Cass County, ND
	DuPont/Pioneer	17	46A65	Lake County, MT
		18	46A76	Lake County, MT

*Company seed lot designations available upon request.

randomly selected two for testing (Table 2-1). Many of the chosen varieties were recommended for other locations in Iowa or Illinois and other states. We deemed it impracticable to try to determine which varieties were the most widely recommended.[27]

For canola, we adopted a slightly different approach, focusing on varieties offered to growers in North Dakota, which accounted for 89 percent of U.S. canola acreage in 2002.[28] Seed companies' websites did not provide specific recommendations for that state, but North Dakota State University provided data on 2001 performance trials of 33 traditional canola varieties. Using these data, we selected five non-engineered varieties from three companies that performed well in the trials[29] (Table 2-1). Assuming that seeds of better-performing varieties would make up a larger proportion of the seed supply than poorly performing varieties, we believe this strategy allowed

27 Company websites are set up in such a way that it is difficult to determine how widely a particular variety is recommended. To do so would require searching for varieties recommended in every crop-growing county or zip code in the country.

28 USDA NASS. 2003. Crop Production: 2002 Summary. Publication CrPr2-1(03), p. 31. On the USDA NASS website at *http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bban/cropan03.pdf*, accessed on November 25, 2003.

29 Like many land-grant universities, North Dakota State University (NDSU) provides information to state growers on the performance of crop varieties as an aid in choosing which varieties to plant. An NDSU Extension Service publication provided data on 33 traditional varieties tested in variety trials in 2001. (NDSU. 2002. 2001 Canola Variety Trials. NDSU Extension Service publication A-1124 [revised], compiled by Duane R. Berglund. Fargo, ND: NDSU, January, p. 1.)

Basics of plant genetic engineering

Genes are functional segments of DNA located on chromosomes within the cells of organisms, including plants. An organism's DNA, comprised of thousands of genes, forms the blueprint for its inherited traits. The full set of genes and associated DNA of an organism is referred to as its **genome**.

Genes code for **proteins**,* the building blocks of organisms. Proteins, working alone or in combination, are responsible for the traits exhibited by plants (e.g., height, flower color, drought tolerance, insect resistance, nutritional makeup). **Regulatory sequences** control the process by which plant cells manufacture proteins. For example, **promoters** are regulatory sequences that operate like switches to start the manufacturing process for a particular protein. They also determine the amount of protein produced. **Genetic sequences** or **elements** refer to genes, regulatory sequences, or pieces thereof.

Genetic engineering involves the use of sophisticated molecular methods to synthesize novel combinations of regulatory sequences and genes and transfer them into an organism. These techniques may be used to transfer genetic sequences between unrelated organisms—from soil bacteria to a corn plant, for example—or to remove and rearrange genetic sequences within a species. Applying these techniques to crops, scientists create crop varieties with new traits. Various terms are used to describe plants produced by these techniques: **genetically engineered**, **genetically modified**, or **transgenic**.

A variety is a subgroup of plants within a crop whose genetic makeup and agricultural characteristics distinguish it from other varieties of that crop. Seed companies are constantly developing new varieties with traits important to growers, such as higher yield or increased resistance to insects and herbicides. These traits may be obtained through genetic engineering or traditional breeding.

To introduce a new trait through genetic engineering, scientists first assemble a **construct**, which can be visualized as a cassette of genetic sequences often taken from several different organisms. Constructs typically carry several regulatory sequences and genes.

After all the pieces of DNA are joined together, the construct is inserted as a unit into an individual plant, creating what scientists refer to as a **transformation event**, or **event** for short. Companies often use the same designation, such as GTS 40-3-2, for both the construct and the plant (and its progeny) created with that construct. A list of events relevant to this report is included below.

As a first step, scientists typically insert new constructs into plant varieties that are easily engineered.

Genetically Engineered Transformation Events

Event	Trade Name	Crop	Company	Trait
176	KnockOut NaturGard	Corn	Syngenta Dow/Mycogen	Resistant to certain insects (expresses Bt toxin)
Bt11	YieldGard†	Corn	Syngenta	Resistant to certain insects (expresses Bt toxin)
CBH-351	StarLink	Corn	Bayer	Resistant to certain insects (expresses Bt toxin)
DBT418	BtXtra	Corn	Monsanto	Resistant to certain insects (expresses Bt toxin)
GA21	Roundup Ready	Corn	Monsanto	Resistant to glyphosate herbicides
GT73	Roundup Ready	Canola	Monsanto	Resistant to glyphosate herbicides
GTS 40-3-2	Roundup Ready	Soybean	Monsanto	Resistant to glyphosate herbicides
MON810	YieldGard†	Corn	Monsanto	Resistant to certain insects (expresses Bt toxin)
NK603	Roundup Ready	Corn	Monsanto	Resistant to glyphosate herbicides
T14 and T25	LibertyLink	Corn	Bayer	Resistant to glufosinate herbicides

SOURCE: AGBIOS website (*http://www.agbios.com*), accessed on September 30, 2003.
†Both Syngenta and Monsanto use Monsanto's registered trademark YieldGard for their respective Bt corn events (Bt11 and MON810).

Transferring the new event into agronomically valuable varieties is accomplished by **traditional plant breeding**.

The diagrams to the right illustrate a generalized construct and a specific construct used to produce soybean varieties resistant to glyphosate herbicides.

GTS 40-3-2 is a construct developed by Monsanto to create Roundup Ready soybeans, which are resistant to the company's glyphosate (Roundup) herbicides. The construct contains a gene coding for a protein and three regulatory sequences: a promoter, a terminator, and a chloroplast transit peptide that directs the new protein to chloroplasts, where it functions in a particular metabolic pathway.

** Some scientists use the term gene to encompass the DNA sequences coding for regulatory sequences as well as proteins.*

A Generalized Construct Used in Genetic Engineering

Regulatory Sequences	Genes for Traits of Interest	Regulatory Sequences
Promoter	Bt insect resistance or herbicide resistance	Terminator

A Specific Construct Conferring Herbicide Resistance in Soybeans (GTS 40-3-2)

Regulatory Sequences		Gene for Herbicide-Resistance Trait	Regulatory Sequences
35S promoter from cauliflower mosaic virus	CTP (chloroplast transit peptide) from petunia	EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene from *Agrobacterium tumefaciens* CP4	NOS terminator from *Agrobacterium tumefaciens*

SOURCE: AGBIOS database product description, MON-04032-6 (GTS 40-3-2). On the AGBIOS website at *http://www.agbios.com/dbase.php?action+showprod&data+gts+40-3-2&frmat=long*, accessed on September 30, 2003.

us to look at a representative sample of a substantial portion of the non-engineered canola seed supply. Because of difficulties in finding seed of the better-performing varieties, we selected one variety (Topscore) that was not part of the 2001 variety trials.

Buying seeds

We bought seeds of all varieties from seed retailers just as growers do. A UCS employee or consultant ordered a bag (approximately 50 pounds) of each variety from seed sellers by phone or in person. The seed sellers shipped the seeds to UCS's Washington, DC, office or a UCS employee or consultant picked up the seeds from the sellers and shipped them by United Parcel Service or delivered them by private vehicle to UCS.

Upon arrival, bags were checked for tears (all arrived with seed bags intact) and were stored in a vacant room within secure UCS offices.

The testing laboratories

To determine whether the seeds contained genetic sequences that might have derived from commercially available engineered varieties, we sent them to two independent, well-established commercial laboratories: GeneScan USA, Inc., and Biogenetic Services, Inc. Both labs specialize in what has come to be called GMO testing—the analysis of food, feed, and other agricultural products to detect sequences from genetically modified organisms (see box, "Basics of plant genetic engineering"). We chose these two companies because of their extensive experience in

GMO testing, their scientists' detailed knowledge and expertise, and their excellent performance in the USDA Grain Inspection, Packers and Stockyards Administration proficiency tests.[30]

GeneScan USA was established five years ago in Belle Chasse, LA, as a subsidiary of GeneScan Europe, AG, which began GMO testing in 1995.[31] GeneScan Europe has a global network of genetic testing labs in North and South America, Europe, Asia, and Australia. Biogenetic Services is a small, privately owned company founded 15 years ago in Brookings, SD. Despite its small size, Biogenetic Services serves a wide array of customers: government agencies, food and seed companies, elevator operators, insurance companies, law firms, and private individuals.[32]

By submitting samples to two independent companies, we increased our confidence in our overall conclusions. Even so, that confidence is tempered by the recognition that GMO testing is still in its infancy and, unlike older, well-established areas of analysis, has neither standardized protocols and reference materials nor a uniform, worldwide system of laboratory accreditation.[33] In light of the uncertainties associated with GMO testing methods and the relatively small number of samples for each crop, our primary focus in this study was determining the presence or absence of engineered sequences. While some of the assays did provide information on the levels at which engineered sequences were found in samples, we do not believe the data are sufficiently robust to draw conclusions about the likely levels of contamination in the seed supply.

We conducted two rounds of testing. In Round One, the first laboratory (GeneScan) assayed seed samples of corn, soybeans, and canola to determine the presence of sequences derived from transgenic crops, estimate the levels of contaminants, and run controls for false positives. In Round Two, the second laboratory (Biogenetic Services) tested seeds of the three crops to confirm the first round tests and assayed a duplicate, but larger, sample of seeds to increase the chances of detecting contaminants.[34] Both laboratories employed widely used testing methods based on polymerase chain reaction (PCR) to detect and identify engineered genetic sequences in the seeds.

Testing method: polymerase chain reaction

Reduced to the simplest terms, PCR testing methods home in on particular target sequences of DNA and, using a special DNA-copying enzyme (DNA polymerase), selectively make enough copies of the target sequence to allow it to be identified and measured. In practice, PCR methods are complicated and require highly trained personnel, sophisticated machinery, and carefully

30 For more information on the USDA Grain Inspection, Packers and Stockyards Administration (GIPSA) program, see the USDA GIPSA website at *http://www.usda.gov/gipsa/biotech/proficiency-program.htm*.

31 For more information on GeneScan USA, Inc., see the GeneScan website at *http://www.gmotesting.com.*

32 For more information on Biogenetic Services, Inc., see the Biogenetic Services website at *http://www.biogeneticservices.com*. Also, see examples of Biogenetic Services' clients at: Plant Genome Database—Prototype Developing (at *http://www.nal.usda.gov/pgdic/probe/v1n3_4/ maize.html*, accessed on September 23, 2003); Progress in the Development of a Genomic RFLP Map of Cultivated Sunflower (*Helianthus annus*) (at *http://www.intl-pag.org/1/abstracts/101pg1.html*, accessed on September 23, 2003); and Conclusions from a Meeting to Discuss the Interpretation of Test Results on Seed Grown at the Affected Sites in Gisborne and Pukekohe, September 18, 2002 (at *http://www.maf.govt.nz/biosecurity/imports/plants/papers/gm-seeds/appendix-10.htm*, accessed on September 23, 2003).

33 Anklam, E., P. Heinze, S. Kay, and G. Van den Eede. 2002. Validation studies and proficiency testing. *Journal of AOAC International* 85(3):809-815.

34 Sample size is a critical factor in the capacity for PCR methods to detect and measure target DNA. Larger samples increase the chances that a given target molecule will be detected and that the amount of the target measured in the sample will be close to the actual amount in the lot from which the sample was taken. For more information on the role of sample size in GMO testing, see Fagan, J. 2004. Detection and Quantification of GMOs by DNA-Based and Protein-Based Methods. Chapter in *Handbook of Food Analysis, second edition,* Marcel Dekker, Inc., in press; Spiegelhalter, F., F.-R. Lauter, and J.M. Russell. 2001. Detection of genetically modified food products in a commercial laboratory. *Journal of Food Science* 66:634-640; USDA GIPSA. 2000. Sampling for the Detection of Biotech Crops. On the USDA GIPSA website at *http://www.usda.gov/gipsa/biotech/sample2.htm*, accessed on November 13, 2001.

Designations for regulatory sequences and genes

CTP2/EPSPS CP4	Sequences characteristic of various glyphosate-resistant (Roundup Ready) crops (see the figure, "A Specific Construct Conferring Herbicide Resistance in Soybeans," p. 17)
hmgA	High-mobility group A, a corn-specific gene
le1	Lectin, a soybean-specific gene
nptII	An antibiotic-resistance gene often used as a selective marker in plant engineering
P35S	A promoter from the cauliflower mosaic virus; widely used in transgenic plants
PFMV	A promoter from the figwort mosaic virus
pepC	Phosphoenol pyruvate carboxylase, a canola-specific gene
T-NOS	A terminator sequence (nopaline synthase) widely used in engineered plants

SOURCE: AGBIOS website (*http://www.agbios.com*).

designed tests incorporating many controls and reference standards to ensure accurate and reproducible results.[35]

Primers, or primer sets, are a key feature of PCR testing methods; they "find" the targeted DNA in a mixture of DNA molecules. Primers are short pieces of DNA synthesized to match sequences at the beginning and end of a segment of targeted DNA. When added to a mixture of DNA molecules extracted from a seed sample, the primers bind to the corresponding beginning and ending segments of the target DNA, thereby marking the exact segment to be copied by the DNA polymerase.

The next step, copying the target DNA, involves a series of different reactions, each requiring a different temperature. Thermocyclers subject mixtures of sample DNA, primer sets, DNA polymerase, and other reagents to a carefully controlled regimen of temperature changes—allowing each of the required reactions to proceed under optimal conditions. Each cycle through the temperature regimen doubles the number of target DNA segments, leading quickly to billions of copies.[36]

Companies use thermocyclers in conjunction with other analytical equipment to generate useful information about the accumulated DNA copies and, by extrapolation, the original sample. GeneScan employed both a qualitative PCR system that determined whether engineered sequences were present or absent in seed samples and a quantitative PCR system to estimate the level of engineered DNA in a sample. Biogenetic Services used a semi-quantitative PCR system that simultaneously detected and estimated the proportion of engineered sequences.

Background on testing strategy

In the study, PCR methods were used for three purposes: to screen for the presence of transgenically derived sequences in the traditional seed samples, to identify the specific transgenic events that were the likely sources of the contaminants, and to estimate the level at which transgenic sequences were present.

Screening for transgenically derived sequences. Screening tests were conducted to determine whether any sequences derived from genetically

35 For more detail on PCR techniques, assay design, controls, reference standards, and interpretation of results, see Fagan, J. 2004 and Spiegelhalter, F. et al. 2001.

36 Spiegelhalter, F. et al. 2001. Theoretically, after 32 cycles, a single target molecule would yield just over one billion copies. In actuality, more cycles would be required because each cycle, for various reasons, usually yields less than a doubling.

engineered crops were present in the seed samples. Most of the corn and soybean events currently on the market were engineered with—and therefore likely to contain—either P35S or T-NOS (see box, "Designations for regulatory sequences and genes," p. 19), so primers for those regulatory sequences were used in the initial screen. By probing for those common regulatory sequences, the tests cast a wide net for potential contaminants.

In contrast to corn and soybean events, not all canola events contain P35S and/or T-NOS, so additional primer sets were used to canvas for the presence or absence of canola constructs. In addition to P35S and T-NOS, GeneScan used nptII and CTP2/EPSPS CP4, and Biogenetic Services used PFMV.

Identifying specific transgenic events. In samples testing positive for transgenically derived sequences in the screening assays, subsequent tests were undertaken to identify the specific engineered events. Our approach for identifying these events was slightly different in different crops.

In soybeans, only one commercial event was likely to have contaminated traditional seeds: Monsanto's Roundup Ready soybeans. Even though the U.S. government has allowed two other engineered soybean events on the market (Bayer's glufosinate-resistant soybeans and DuPont's altered-oil soybeans), these events are planted on little, if any, acreage and are less likely to contaminate traditional soybeans. In first-round screening assays, we assumed the genetic sequences detected in soybean samples using primers for P35S and T-NOS came from Roundup Ready (event GTS 40-3-2). Quantitative tests conducted in the first round confirmed that assumption.

Canola seeds testing positive for transgenically derived sequences were assayed for the presence of only one engineered canola event—Monsanto's Roundup Ready (event GT73)—even though other events have been commercialized. Neither lab had the primer sets necessary to assay Bayer's LibertyLink and SeedLink or Monsanto's Laurical.

Primer sets for many Bt corn events are available to laboratories. The corn samples testing positive for transgenically derived sequences were subjected to additional PCR tests to identify which commercial engineered corn events might be the source of the contaminating DNA. The two laboratories in this study used primer sets recognizing specific commercial corn events such as 176, Bt11, and MON810.

The identification of specific events in this study helped confirm that the genetic sequences detected in screening tests did indeed originate in engineered varieties and ruled out "other seeds" (for example, corn seeds in bags of soybean seed) as major sources of false positive results.

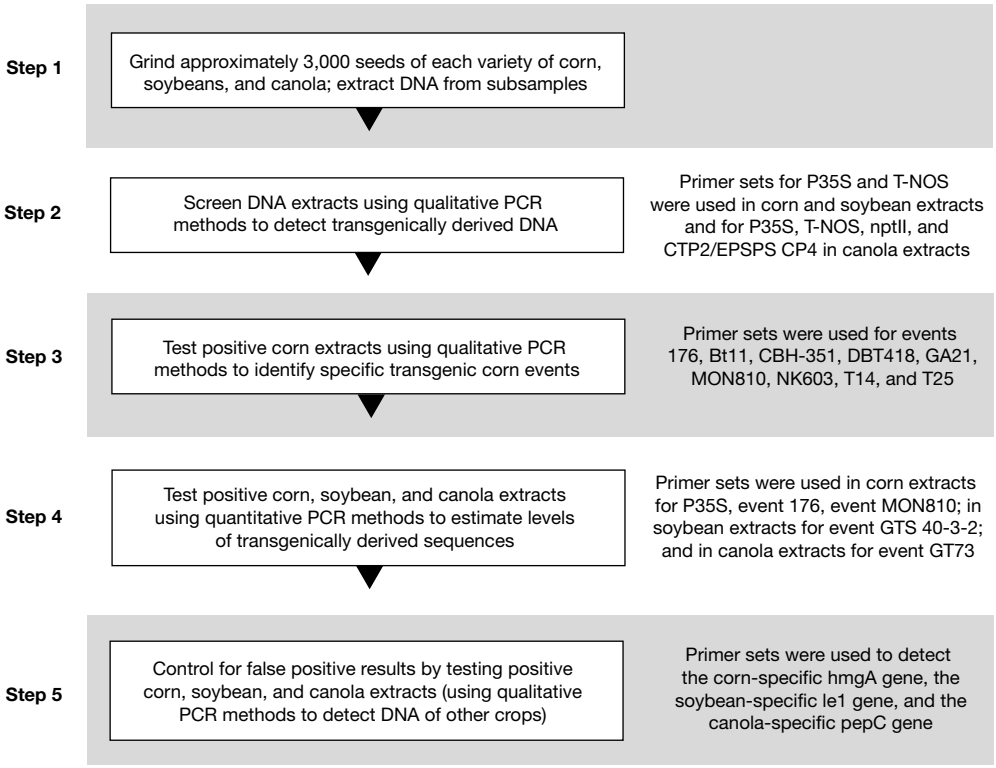
Estimating the levels of transgenically derived sequences. GeneScan and Biogenetic Services provided data on the percentage of genomes in the samples that carried transgenically derived sequences (i.e., the number of genomes containing target DNA detected in comparison to the total number of crop genomes detected in a seed sample times 100). For example, a PCR test detecting 2,000 genomes of Roundup Ready varieties and 1,000,000 genomes of soybean DNA in a sample would report 0.2 percent Roundup Ready DNA.[37]

Round One testing

In Round One, GeneScan tested seed samples from each of six varieties of corn, soybeans, and canola. We weighed, packaged, and shipped approximately 2.5 pounds of seeds of each variety, taking special precautions to prevent cross-contamination of varieties.

37 For more information on quantifying DNA, see Spiegelhalter, F. et al. 2001.

Figure 2-1 **Round One: Detecting and Estimating the Levels of Transgenically Derived DNA (3,000-Seed Samples)**



Detecting transgenically derived DNA. The laboratory ground approximately 3,000 seeds[38] of each variety, extracted DNA from a subsample of the ground material, and used qualitative PCR methods to screen DNA samples. As shown in Figure 2-1, Step 2, primer sets for P35S and T-NOS were used to screen corn and soybean samples, and P35S, T-NOS, nptII, and CTP2/EPSPS CP4 were used to screen canola samples (at a detection limit of approximately 0.1 percent[39]).

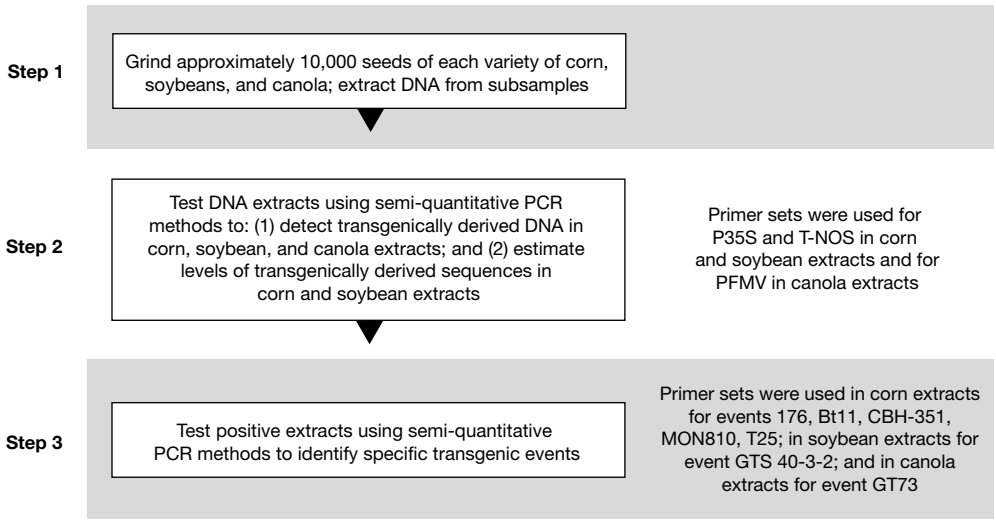
Determining specific transgenic events. For samples testing positive for transgenically derived sequences, the laboratory used qualitative PCR methods to determine which specific events might be the source of the contaminating DNA. As explained above, positive corn samples were subjected to further PCR testing to distinguish among a number of commercial engineered events (Figure 2-1, Step 3), and we assumed positive soybean extracts were contaminated with DNA from Roundy Ready (event GTS 40-3-2) soybeans. Canola extracts were subjected to PCR using a primer set for one canola event: GT73 (Roundup Ready).

Estimating the levels of transgenically derived sequences. After determining the presence or absence of regulatory and gene sequences, the

38 The company weighed the equivalent of approximately 3,000 seeds, based on data on weights of aliquots of known numbers of seeds.

39 A 0.1 percent detection limit means that the methods could not reliably detect target DNA if it were present in the samples at less than a 0.1 percent level.

Figure 2-2 **Round Two: Detecting and Estimating the Levels of Transgenically Derived DNA (10,000-Seed Samples)**



laboratory used quantitative PCR methods to estimate the percentage of genomes that carried transgenically derived sequences in positive seed samples (typically at an approximate quantification limit of 0.05 percent[40]). Figure 2-1, Step 4 (p. 21) shows the primer sets used in each crop.

Reducing false positive results. Genetically engineered varieties of crops other than the one being tested are potential sources of false positive results. For example, a false positive result in soybean seeds might be the result of contamination with engineered corn seed. We attempted to eliminate this possibility by visually inspecting the samples for seeds of other crops before shipping. Nonetheless, contaminating seeds or pieces of seed remained a possibility.

To determine whether the seed samples were contaminated by engineered sequences derived from other crops, the laboratory assayed positive samples for the presence of DNA from two other crops for which transgenic varieties have been allowed on the market. Using primers for genes unique to each crop, corn samples were tested for the presence of canola and soybean DNA, soybean for corn and canola DNA, and canola for corn and soybean DNA. (See Figure 2-1, Step 5, p. 21, for primer sets used to detect crop-specific DNA.)

In addition, naturally occurring plant viruses in canola seeds may yield positive results for P35S. The laboratory avoided this potential outcome by using primers for sequences in addition to P35S when testing canola (Figure 2-1, Step 2, p. 21).

Round Two testing

Biogenetic Services tested additional and larger samples of corn, soybean, and canola seeds taken from the same 50-pound bags used in the first round of tests. These second-round tests were undertaken to confirm GeneScan's results and to determine whether larger samples of seeds would increase the likelihood of obtaining a positive

40 A 0.05 percent quantification limit means that the methods could not reliably measure target DNA if it were present in the samples at less than a 0.05 percent level.

Table 2-2 **Round One Results: Presence and Levels of Transgenically Derived DNA**

Crop	Variety Designation*	Transgenically Derived DNA Detected	Transgenic Events Detected	% of Total Genomes Containing Transgenically Derived DNA**
Corn	1	No	None	None
	2	No	None	None
	3	Yes	MON810 (YieldGard)	Less than 0.05%
	4	No	None	None
	5	Yes	MON810 (YieldGard)	0.1%
	6	Yes	176 (KnockOut/NaturGard) MON810 (YieldGard)	Less than 0.2% Less than 0.05%
Soybean	7	No	None	None
	8	No	None	None
	9	Yes	GTS 40-3-2 (Roundup Ready)	Less than 0.05%
	10	Yes	GTS 40-3-2 (Roundup Ready)	Less than 0.05%
	11	Yes	GTS 40-3-2 (Roundup Ready)	Less than 0.05%
	12	No	None	None
Canola	13	Yes	GT73 (Roundup Ready)	Less than 0.05%
	14	Yes	GT73 (Roundup Ready)	0.05%
	15	Yes	GT73 (Roundup Ready)	0.05%
	16	Yes	GT73 (Roundup Ready)	0.1%
	17	Yes	GT73 (Roundup Ready)	0.1%
	18	Yes	GT73 (Roundup Ready)	Less than 0.05%

*See Table 2-1, p. 15.
**Limit of quantification = 0.05% except for event 176 (0.2%).

result from any one 50-pound bag, thus providing a more accurate picture of the extent of contamination.

We weighed, packaged, and shipped approximately nine, seven, and three pounds of seeds of each variety of corn, soybeans, and canola, respectively, to the second laboratory using the same protocol, except for sample size, as with the first laboratory. We shipped enough seeds to grind 10,000 seeds of each variety (compared with the 3,000 seeds ground by the first laboratory). The seeds of each variety sent to the second laboratory were scooped from the same bag sampled for first-round testing.

Detecting and estimating the levels of transgenically derived sequences. Biogenetic Services ground approximately 10,000 seeds[41] of each variety of corn and soybeans and extracted DNA from a subsample of the ground material. Using semi-quantitative PCR methods, the laboratory screened DNA samples with primer sets for the common regulatory sequences P35S and T-NOS and estimated the levels of transgenically derived sequences in positive samples (at detection and quantification limits of approximately 0.1 percent). The same process was followed for canola seeds, except the laboratory screened with primer sets for PFMV and did not estimate the levels of transgenically derived sequences (Figure 2-2, Steps 1 and 2).

Determining specific transgenic events. To determine which specific events might be responsible for the contamination, positive samples of corn, soybean, and canola seeds were subjected

41 The company weighed the equivalent of approximately 10,000 seeds, based on data on weights of aliquots of known numbers of seeds.

Table 2-3 **Round Two Results: Presence and Levels of Transgenically Derived DNA**

Crop	Variety Designation*	Transgenically Derived DNA Detected	Transgenic Events Detected	% of Total Genomes Containing Transgenically Derived DNA**
Corn	1	Yes	Bt11 (YieldGard) MON810 (YieldGard)	Between 0.5 and 1.0%
	2	Yes	176 (KnockOut/NaturGard) MON810 (YieldGard) T25 (LibertyLink)	Approximately 1.0%
	3	Yes	176 (KnockOut/NaturGard) Bt11 (YieldGard) MON810 (YieldGard)	Approximately 1.0%
	4	No	None	None
	5	Yes	176 (KnockOut/NaturGard) Bt11 (YieldGard) MON810 (YieldGard)	Approximately 1.0%
	6	Yes	176 (KnockOut/NaturGard) MON810 (YieldGard)	Approximately 1.0%
Soybean	7	Yes	GTS 40-3-2 (Roundup Ready)	Between 0.5 and 1.0%
	8	No	None	None
	9	Yes	GTS 40-3-2 (Roundup Ready)	More than 1.0%
	10	Yes	GTS 40-3-2 (Roundup Ready)	More than 1.0%
	11	Yes	GTS 40-3-2 (Roundup Ready)	More than 1.0%
	12	Yes	GTS 40-3-2 (Roundup Ready)	Between 0.1 and 0.5%
Canola	13	Yes	GT73 (Roundup Ready)	QND***
	14	Yes	GT73 (Roundup Ready)	QND
	15	Yes	GT73 (Roundup Ready)	QND
	16	Yes	GT73 (Roundup Ready)	QND
	17	Yes	GT73 (Roundup Ready)	QND
	18	No	None	QND

*See Table 2-1, p. 15.
**Limit of quantification = 0.1%. Estimates were made of the total transgenically derived DNA detected using P35S and T-NOS, not of individual events.
***Quantification not done.

to additional semi-quantitative PCR tests (Figure 2-2, Step 3, p. 22). This time, primers for specific engineered events were used (at a detection limit of approximately 0.1 percent).

RESULTS

Overall, the pilot study showed that seeds of traditional varieties of corn, soybeans, and canola are contaminated at a high incidence with low levels of genetic sequences derived from transgenic crop varieties.

Incidence of contamination

Round One results. Transgenically derived sequences were detected in seeds of three of six traditional varieties (50 percent) of corn and soybeans and in all six traditional varieties (100 percent) of canola (Table 2-2, p. 23). Monsanto events were detected in all three crops: MON810 (YieldGard) in the three contaminated corn varieties, GTS 40-3-2 (Roundup Ready) in the three contaminated soybean varieties, and GT73 (Roundup Ready) in all six contaminated canola varieties. Syngenta's event 176 (KnockOut/NaturGard) was detected in one contaminated corn variety.

Round Two results. Transgenically derived sequences were detected in seeds of five of six traditional varieties (83 percent) of all three crops (Table 2-3). Of the five contaminated corn varieties, three

Table 2-4 **Combined Results of Rounds One and Two: Presence and Levels of Transgenically Derived DNA***

Crop	Variety Designation**	Transgenically Derived DNA Detected		Transgenic Events Detected		% of Total Genomes Containing Transgenically Derived DNA**	
		Round 1	Round 2	Round 1	Round 2	Round 1***	Round 2****
Corn	1	No	Yes	None	Bt11 (YieldGard) MON810 (YieldGard)	None	Between 0.5 and 1.0%
	2	No	Yes	None	176 (KnockOut/NaturGard) MON810 (YieldGard) T25 (LibertyLink)	None	Approximately 1.0%
	3	Yes	Yes	MON810 (YieldGard)	176 (KnockOut/NaturGard) Bt11 (YieldGard) MON810 (YieldGard)	Less than 0.05%	Approximately 1.0%
	4	No	No	None	None	None	None
	5	Yes	Yes	MON810 (YieldGard)	176 (KnockOut/NaturGard) Bt11 (YieldGard) MON810 (YieldGard)	0.1%	Approximately 1.0%
	6	Yes	Yes	176 (KnockOut/ NaturGard) MON810 (YieldGard)	176 (KnockOut/NaturGard) MON810 (YieldGard)	Less than 0.2%*** Less than 0.05%	Approximately 1.0%
Soybean	7	No	Yes	None	GTS 40-3-2 (Roundup Ready)	None	Between 0.5 and 1.0%
	8	No	No	None	None	None	None
	9	Yes	Yes	GTS 40-3-2 (Roundup Ready)	GTS 40-3-2 (Roundup Ready)	Less than 0.05%	More than 1.0%
	10	Yes	Yes	GTS 40-3-2 (Roundup Ready)	GTS 40-3-2 (Roundup Ready)	Less than 0.05%	More than 1.0%
	11	Yes	Yes	GTS 40-3-2 (Roundup Ready)	GTS 40-3-2 (Roundup Ready)	Less than 0.05%	More than 1.0%
	12	No	Yes	None	GTS 40-3-2 (Roundup Ready)	None	Between 0.1 and 0.5%
Canola	13	Yes	Yes	GT73 (Roundup Ready)	GT73 (Roundup Ready)	Less than 0.05%	QND*****
	14	Yes	Yes	GT73 (Roundup Ready)	GT73 (Roundup Ready)	0.05%	QND
	15	Yes	Yes	GT73 (Roundup Ready)	GT73 (Roundup Ready)	0.05%	QND
	16	Yes	Yes	GT73 (Roundup Ready)	GT73 (Roundup Ready)	0.1%	QND
	17	Yes	Yes	GT73 (Roundup Ready)	GT73 (Roundup Ready)	0.1%	QND
	18	Yes	No	GT73 (Roundup Ready)	None	Less than 0.05%	QND

*3,000 and 10,000 seeds of each variety were tested in Round One and Round Two, respectively.
**See Table 2-1, p. 15.
***Limit of quantification = 0.05% except for event 176 (0.2%).
****Limit of quantification = 0.1%.
*****Quantification not done.

contained three transgenic events and two contained two events. Monsanto events were detected in all three crops: MON810 (YieldGard) in the five contaminated corn varieties, GTS 40-3-2 (Roundup Ready) in the five contaminated soybean varieties, and GT73 (Roundup Ready) in the five contaminated canola varieties. Syngenta events 176 (KnockOut/NaturGard) and Bt11 (YieldGard) were detected in four and three contaminated corn varieties, respectively. Bayer event

T25 (LibertyLink) was detected in one corn variety.

Combined results. The positive results in the first round were largely confirmed and extended by second-round tests (Tables 2-4, p. 25, and 2-5). The second laboratory, which used a larger seed sample (10,000 versus 3,000), found a higher incidence of engineered contaminants in corn and soybeans and a lower incidence in canola. In addition, the second laboratory found a larger number of contaminating events in corn varieties than the first. The most conservative expression of the combined results is that transgenically derived DNA was detected in 50 percent of the corn, 50 percent of the soybeans, and 83 percent of the canola varieties tested.

Table 2-5 **Combined Results of Rounds One and Two: Percentage of Tested Varieties Containing Transgenically Derived DNA**

Crop	Number and % of Tested Traditional Varieties Containing Transgenically Derived DNA*			
	Round 1 (3,000 seeds)		Round 2 (10,000 seeds)	
	Number	%	Number	%
Corn	3 of 6	50	5 of 6	83
Soybean	3 of 6	50	5 of 6	83
Canola	6 of 6	100	5 of 6	83

*See text and Table 2-4, p. 25, for more detail.

Estimated levels of contamination

Round One results. In contaminated corn varieties, MON810-derived sequences were estimated at levels ranging from 0.1 percent to less than 0.05 percent of the corn genomes present and event 176 was found in one variety at less than 0.2 percent. In all three contaminated soybean varieties, GTS 40-3-2 was estimated to be less than 0.05 percent of the soybean genomes present. The six canola varieties were contaminated with GT73 at estimated levels ranging from 0.1 percent to less than 0.05 percent of the canola genomes present. All Round One assays had a quantification limit of 0.05 percent except for event 176, for which the limit was 0.2 percent (Table 2-2, p. 23).

Round Two results. The second laboratory estimated the levels of transgenically derived sequences in corn and soybean samples based on the total transgenically derived DNA detected by primers for common regulatory sequences. It did not, however, quantify individual events in corn as did the first laboratory (Table 2-3, p. 24).

In four of five contaminated corn samples, approximately one percent of the corn genomes present contained transgenically derived sequences, while the fifth sample was slightly less contaminated, at less than one percent but more than 0.5 percent of the corn genomes present. In soybeans, the laboratory determined that more than one percent of the soybean genomes in three varieties contained transgenically derived sequences. The remaining two varieties had lower levels of transgenic genome contamination, ranging between 0.1 and 1 percent. All Round Two assays had a quantification limit of 0.1 percent. The second laboratory did not run quantitative assays for contaminants in canola.

Combined results. In the samples where transgenically derived DNA was detected, the percentage of total genomes containing transgenically derived sequences ranged from less than 0.05 percent to approximately one percent in corn, less than 0.05 percent to more than one percent in soybeans, and less than 0.05 to 0.1 percent in canola.

Overall, the estimated levels of transgenically derived sequences in contaminated traditional seeds of the three crops ranged from less than 0.05 percent of the total genomes present in the samples to more than one percent. As discussed above, PCR methodology is still in its infancy and lacks standard protocols and methods. As a result, it is difficult to combine data from different laboratories. While we have presented data from quan-

Table 2-6 **Round One Tests for False Positives**

Crop	Designations of Varieties Testing Positive for Transgenically Derived DNA*	Presence (+)/Absence (-) of Other-Crop DNA
Corn	3	Soybean - Canola -
	5	Soybean - Canola -
	6	Soybean - Canola -
Soybean	9	Corn - Canola -
	10	Corn + Canola -
	11	Corn + Canola -
Canola	13	Corn - Soybean -
	14	Corn - Soybean -
	15	Corn - Soybean -
	16	Corn - Soybean -
	17	Corn - Soybean +
	18	Corn - Soybean -

*See Table 2-1, p. 15.

titative and semi-quantitative analyses of the seed samples we had tested, we do not believe these data are robust enough to draw conclusions about the levels of contamination in the seed supply.

We note that the second laboratory, which tested 10,000 rather than 3,000 seeds of each variety, reported higher levels of contamination in corn and soybeans than the first laboratory (Table 2-4, p. 25), but we believe different methods and samples make it impossible to interpret this difference. Even preliminary conclusions on the levels of contamination must await a larger study and the development of a standard testing methodology.

Potential false positive results

Of the 12 varieties testing positive for transgenically derived sequences in the first round, three contained the DNA of other crops: two soybean varieties were contaminated with corn DNA and one canola variety was contaminated with soybean DNA (Table 2-6). Therefore, it is possible that engineered seeds from other crop varieties could have contributed to the positive test results on incidence.

However, that source of contamination could not have accounted for all the engineered genetic sequences detected in the tests because assays with specific primers provided independent evidence that contamination originated in varieties of the tested crop. In the two soybean varieties contaminated with corn DNA, some of the transgenic sequences may have come from corn. But PCR methods used to estimate the levels of engineered genetic sequences relied on a primer set specific for transgenic soybean (the Roundup Ready soybean event GTS 40-3-2). That primer set would not have recognized any commercial engineered corn events.

Similarly, in the canola variety contaminated with soybean DNA, the quantitative PCR testing in Round One was conducted with a primer set specific for transgenic canola (the Roundup Ready canola event GT73). That primer set does not recognize the Roundup Ready soybean event.

UNDERSTANDING THE RESULTS

Extent of contamination

As Tables 2-4 (p. 25) and 2-5 show, one laboratory found engineered contaminants in half the corn and soybean varieties and all the canola varieties. The second laboratory found five of six, or 83 percent, of the varieties of all three crops were contaminated with engineered sequences. Although the sample size is small, the sampling methodology we used suggests that the contamination of the traditional seed supply is likely to be pervasive.

The 18 varieties we selected were marketed to farmers in states planting the most corn, soybean,

and canola seeds in the United States. Four of the six seed companies from which we purchased seed are among the biggest in the country, controlling a substantial portion of the U.S. traditional seed supply. So it is likely that these 18 varieties represent a substantial portion of the 2002 traditional seed supply for these three crops.[42]

It seems improbable that all or most of the other varieties we did not test were free of transgenic contaminants.

Expression of new traits

Not all the contaminants detected by the PCR methods in this study would lead to the expression of engineered traits in the plants grown from these seeds. In general, only those seeds containing intact constructs (i.e., the full complement of regulatory and gene sequences needed to confer the trait) will produce a plant exhibiting new characteristics.

Transgenic constructs may fragment and/or rearrange once they are within a plant genome,[43] leading in some instances to separation of regulatory and gene sequences. Genes alone would produce new protein only in the unlikely event that they were positioned in the neighborhood of a resident regulatory sequence. Regulatory sequences by themselves would not be able to produce novel functional proteins. On the other hand, if they were located in proximity to resident genes, the transgenically derived regulatory sequences may be able to alter the level of expression of those genes and perhaps confer new traits.

Routes of contamination

It is worth emphasizing that this study provides no information on how or when the commingling that led to the contamination occurred. The genetic sequences detected in this study could have moved into traditional seeds by either physical mixing or outcrossing, which could have occurred last year or several years ago. The lack of information on the mode and timing of commingling makes it difficult to speculate on just how extensive the contamination is or where in the production or handling of seeds intervention could have prevented it.

Nevertheless, the study does provide one insight into the role of physical mixing. We initially assumed gene flow rather than physical mixing was the likely primary cause of contamination and predicted that transgenic sequences would most likely show up in corn and canola—crops with outcrossing rates well above that of the predominantly self-pollinating soybeans. The results, however, show all but one traditional soybean variety contaminated with transgenically derived DNA, suggesting that seed mixing during seed production and handling—at planting, harvest, processing, storage, or transport—may be able to produce widespread contamination.

Illustration of low levels of contamination

As stated earlier, we are not suggesting this study provides a basis for determining overall levels of contamination. The fact that we detected transgenic sequences in so many samples, however, makes it appropriate to consider what low levels of contaminants in the traditional seed supply might mean in practical terms.

To do that, we converted the percentage of total genomes carrying transgenically derived sequences into a percentage of contaminated seeds and then attempted to visualize contamination in

42 Companies do not release sales data on individual varieties to the public, so we could not determine which varieties were the most widely planted in 2002.

43 Svitashev, S.K., W.P. Pawlowski, I. Makarevitch, D.W. Plank, and D. Somers. 2002. Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement. *The Plant Journal* 32(4):433-445. On the Blackwell-Synergy website at *http://blackwell-synergy.com/links/doi/10.1046/j.1365-313X.2002.01433.x*, accessed on November 6, 2003.

Table 2-7 **Illustration of Low Levels of Seed Contamination**

Crop	Estimated number of seeds of transgenic varieties contaminating seeds of traditional varieties at a level of:		Estimated number of 50-pound bags required to hold seeds of transgenic varieties contaminating seeds of traditional varieties at a level of:		Estimated number of large tractor-trailer trucks* required to hold seeds of transgenic varieties contaminating seeds of traditional varieties at a level of:	
	0.1%	1%	0.1%	1%	0.1%	1%
Corn**	1.6 billion	16 billion	25,000	250,000	24	240
Soybean***	4.4 billion	44 billion	32,000	320,000	31	308
Canola****	270 million	2.7 billion	47	470	Less than 1	Less than 1

*We assumed that a large tractor-trailer truck would have a 26-ton carrying capacity. (Iowa Department of Transportation. 1994. Compare cargo capacity. On the Silos and Smokestacks National Heritage Area website at *http://silosandsmokestacks.org/resources/images/scans/comparedot.gif*, accessed on February 11, 2003.)
**Based on estimates of the number of traditional corn seeds (1.6 trillion) planted in the United States in 2002. See text for more detail.
***Based on estimates of the number of traditional soybean seeds (4.4 trillion) planted in the United States in 2002. See text for more detail.
****Based on estimates of the number of traditional canola seeds (270 billion) planted in North Dakota in 2002. See text for more detail.

three ways: the number of contaminating seeds and the number of 50-pound bags and large (26-ton) tractor-trailer trucks needed to hold the seeds (Table 2-7; Figure 2-3, p. 30; Figure 2-4, p. 31).

For the sake of convenience, we assume that the percentage of genomes translates directly into the percentage of seeds carrying genetically engineered sequences.[44] This study reports percentages of total genomes containing transgenically derived sequences ranging from less than 0.05 percent to more than one percent. For the purposes of this exercise, these numbers translate into a range of less than 0.05 percent to more than one percent of the total seeds carrying transgenic sequences in the samples tested.

These levels may appear low, and may lead some to believe that the quantities of seed they represent are small. But that would be a mistake. To emphasize this point, we have estimated the number of contaminating seeds and the number of 50-pound bags and large tractor-trailer trucks required to hold the seeds that 0.1 and 1 percent levels of contamination would represent of the seeds planted with traditional corn, soybean, and canola varieties. For our calculations, we used data on the acreage planted with traditional varieties of each crop in 2002.

Illustrating low levels of contaminants in corn and soybean seeds. Using USDA data on the acreage of traditional crop varieties planted and published information on planting rates (number of seeds per acre), we estimated the number of seeds of traditional varieties of corn and soybeans planted in the United States in 2002 to be roughly 1.6 trillion for corn and 4.4 trillion for soybeans.[45]

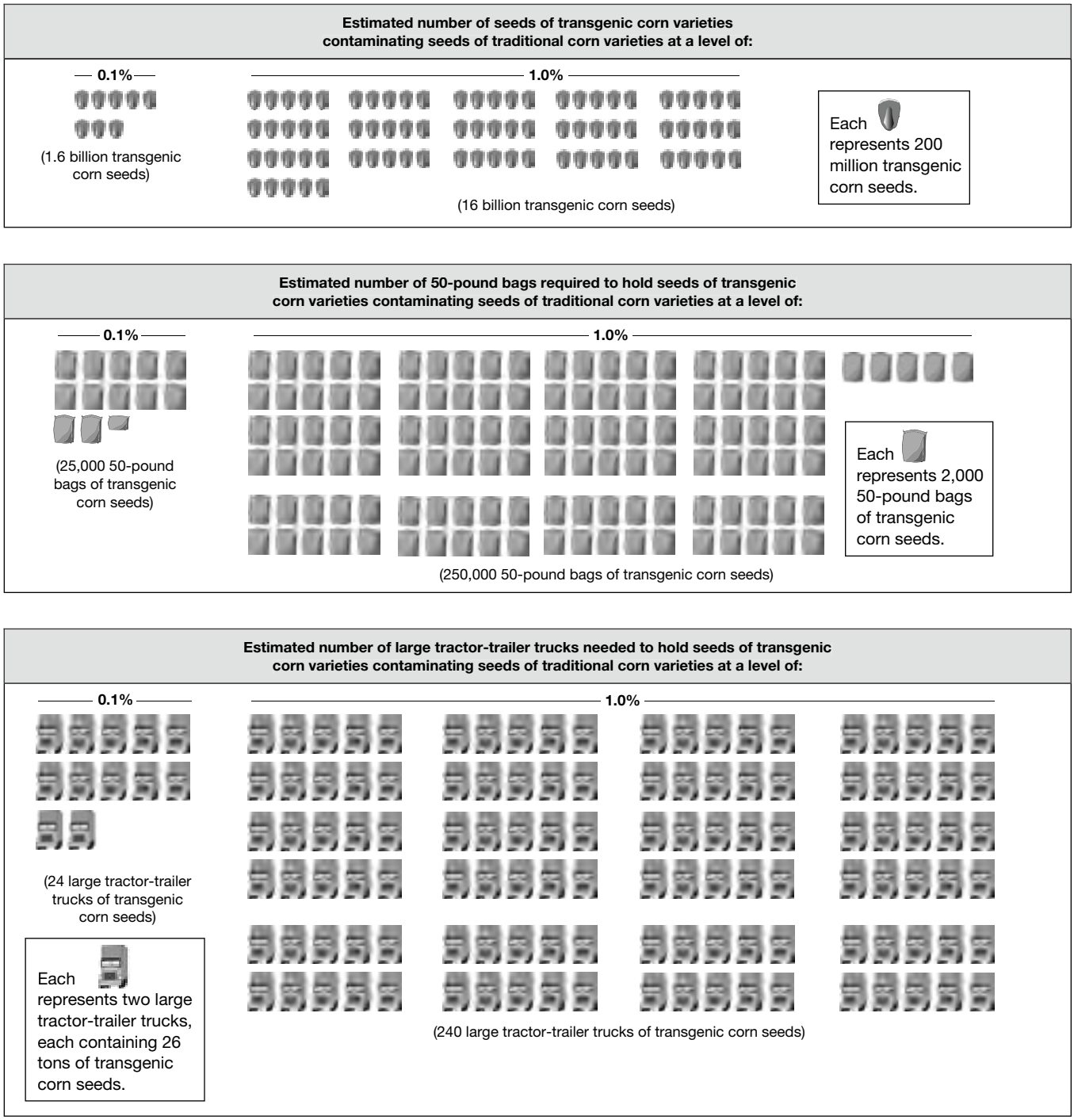
We then calculated the number of seeds carrying transgenic sequences that would have

continued on page 32

44 The conversion of percentage genomes into percentage seeds contaminated is not straightforward because of issues like ploidy (the number of genomes per cell) and zygosity (whether genetic elements were contributed by one or both parents), some of which may be taken into account by GMO testing companies' adding particular PCR controls.

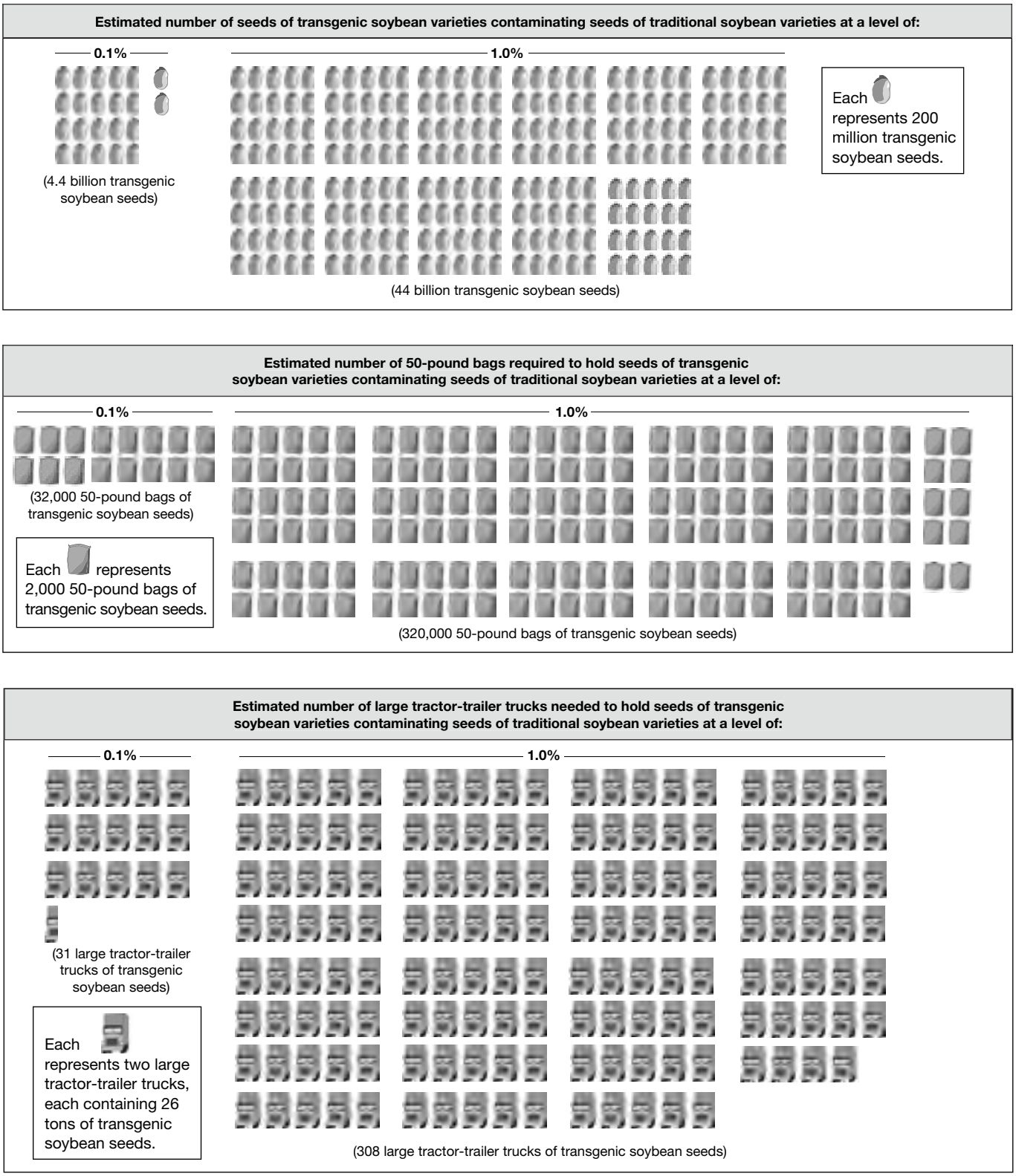
45 According to USDA NASS data, 79,054,000 acres were planted with corn (*http://www.usda.gov/nass/aggraphs/cornacm.htm*) and 73,758,000 acres were planted with soybeans (*http://www.usda.gov/nass/aggraphs/soyacm.htm*) in the United States in 2002 (USDA NASS website accessed on May 15, 2003). Approximately 52 million acres, 66 percent of the total corn acreage, were planted with traditional varieties. Approximately 18 million acres, 25 percent of the total soybean acreage, were planted with traditional varieties in 2002 (USDA NASS website at *http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bbp/pspl0303.pdf* accessed on August 15, 2003). We calculated estimates of planting rates for corn (30,400 seeds per acre) and soybeans (243,000 seeds per acre) from data in Hoeft, R.G., E.D. Nafziger, R.R. Johnson, and S.R. Aldrich. 2000. *Modern Corn and Soybean Production*. Champaign, IL: MCSP Publications, pp. 90-94. Multiplying the traditional acreage for each crop by the estimated planting rate, we arrived at roughly 1.6 trillion and 4.4 trillion seeds of corn and soybean, respectively, planted in traditional varieties in 2002.

Figure 2-3 **Graphic Illustration of Low Levels of Seed Contamination in Corn**



Calculations based on U.S. acreage planted with traditional varieties of corn in 2002. See text and Table 2-7, p. 29, for more detail on calculations.

Figure 2-4 **Graphic Illustration of Low Levels of Seed Contamination in Soybeans**



Calculations based on U.S. acreage planted with traditional varieties of soybeans in 2002. See text and Table 2-7, p. 29, for more detail on calculations.

continued from page 29

been planted in fields of traditional corn and soybean varieties if all traditional seed planted in the United States in 2002 had been contaminated at levels of 0.1 or 1 percent (Table 2-7, p. 29, and Figures 2-3, p. 30, and 2-4, p. 31).

At the 0.1 percent contamination level, 1.6 billion corn seeds carrying transgenic sequences would have been planted in fields of traditional varieties of corn in 2002. At the same contamination level, 4.4 billion soybean seeds carrying transgenic sequences would have been planted in fields of traditional varieties of soybeans.

According to our estimates, it would take about 25,000 50-pound bags (the standard size bought by farmers) or 24 large tractor-trailer trucks to hold the 1.6 billion contaminating corn seeds and 32,000 50-pound bags or 31 large tractor-trailer trucks to hold the 4.4 billion contaminating soybean seeds.[46]

At one percent contamination, 16 billion contaminating corn seeds (or 250,000 50-pound bags or 240 large tractor-trailer trucks) and 44 billion contaminating soybean seeds (or 320,000 50-pound bags or 308 large tractor-trailer trucks) would have been planted along with seeds of traditional varieties.

Illustrating low levels of contaminants in canola seeds. Since the USDA does not publish national data on acres planted with traditional and engineered canola varieties, but that information was available for North Dakota, we limited our estimates of traditional canola seeds planted to that state. Based on North Dakota State University estimates of traditional acreage and published information on canola planting rates, we estimated the number of seeds of traditional canola varieties planted in North Dakota in 2002 to be approximately 270 billion.[47]

We similarly estimated the number of canola seeds carrying transgenic sequences that would have been planted in fields of traditional canola varieties in North Dakota in 2002 at a contamination level of 0.1 or 1 percent. Finally, we estimated the number of 50-pound bags[48] or large tractor-trailer trucks that would be required to hold the contaminating seeds (Table 2-7, p. 29).

At a 0.1 percent contamination level, North Dakota farmers would have planted an estimated 270 million canola seeds containing transgenic sequences, or 47 50-pound bags (less than one tractor-trailer truck), in fields of traditional canola varieties. At a one percent contamination level, 2.7 billion contaminating canola seeds, or 470 50-pound bags (less than one tractor-trailer truck), would have been planted. The 270 million and 2.7 billion canola seeds would weigh approximately 1.2 and 12 tons, respectively.

STUDY LIMITATIONS

This pilot study was limited in three important ways.

First, the study tested for only a subset of the genetic sequences present in engineered varieties of corn, soybeans, and canola. As discussed

46 To calculate the numbers of 50-pound bags and 26-ton tractor-trailer trucks required to hold the corn and soybean seeds, we estimated 1,300 seeds per pound for corn (the National Corn Growers Association website at *http://www.ncga.com/education/main/faq.html#kernels*) and 2,750 seeds per pound for soybeans (Hoeft, R.G. et al. 2000, p. 93).

47 Berglund, D.R. 2003. Personal communication, August 15. D.R. Berglund is a professor and extension agronomist at North Dakota State University. Since the USDA does not publish information on the percentage of canola acres planted with engineered and non-engineered varieties in the United States, we relied on data from North Dakota State University (NDSU). (North Dakota accounted for nearly 90 percent of the total U.S. canola acreage in 2002.) According to Dr. Berglund, approximately 400,000 (or 31 percent) of North Dakota's total of 1,300,00 acres of canola were planted with non-engineered varieties in 2002. We calculated an estimated planting rate for canola of 678,000 seeds per acre from data published by University of Minnesota [UM] Extension Service. 1999. Canola Variety Trials. Publication MR-7348-GO. On at the UM Extension Service website at *http://www.extension.umn.edu/distribution/cropsystems/DC7348.html*, accessed on August 5, 2002. Multiplying the traditional acreage by the estimated planting rate, we arrived at roughly 270 billion seeds of traditional canola varieties planted in North Dakota in 2002.

48 We used an estimate of 113,000 seeds per pound for canola (UM Extension Service, 1999).

above, GMO testing laboratories can only test for sequences for which they have primer sets, and can only obtain or synthesize primer sets for certain engineered events (primarily those that have been used commercially).

The two laboratories tested for some of the most common transgenic sequences, such as the cauliflower mosaic virus promoter and genes for popular herbicide- and insect-resistance events. However, as noted above, there are other events and regulatory sequences allowed in corn, soybeans, and canola for which the testing laboratories did not have primers. Beyond that, there are many transgenes that are either still undergoing, or have undergone, field tests for which primers are unavailable. To the extent that our study did not test for all possible engineered contaminants, it underestimates the degree of contamination.

The field testing of transgenic corn, soybeans, and canola represents a potentially major source of contaminants not assessed by this study. Since 1987, the USDA has received more than 5,500 applications and notifications of field trials for these three crops—and has denied few. Appendix B contains a list of transgenes and transgenic traits from USDA records of field tests allowed in corn, soybeans, and canola during the last 16 years.

Many, if not most, of the crop-transgene combinations listed in Appendix B will not be commercialized, but they are nevertheless potential sources of contaminating transgenes. The total acreage devoted to field testing is difficult to estimate because one USDA record for a field trial may include tests of multiple transgenes at multiple locations over several years. Since plot sizes typically range from a tenth of an acre to hundreds of acres, however, overall acreage over the past decade and a half is likely to have involved thousands of acres. Many of these tests have been carried out in areas of the country where seed production occurs. Thus, it is possible that transgenes from field test plots have migrated to nearby seed production fields in the past and are still doing so today.

Second, the study looked at the commercial seed supply for traditional varieties of only three crops. It did not include other crops such as cotton and squash, which have engineered varieties in commercial use for which laboratories might have obtained primers. To the extent that seeds for traditional varieties of the crops beyond the three we tested are also contaminated, the overall problem is underestimated by our study.

Third, the study methods do not rule out false positives, or contaminants from other engineered crops. The two corn and two soybean varieties that tested positive for transgenically derived sequences in Round Two but not Round One were not tested for contamination by other crops. Since common regulatory sequences were used by Biogenetic Services to estimate the levels of contaminating DNA in samples, these tests might have picked up genetic sequences contributed by other engineered crops, thereby potentially overestimating the level of genetic sequences contributed by engineered events of the original crop.

Because we did not test for DNA sequences from all the crops with commercially approved transgenic varieties, including cotton and squash, there remains a small possibility that some contaminants in positive samples may have come from those crops.

SUMMARY

For this study, UCS staff bought seeds of traditional varieties of three major commodity crops—corn, soybeans, and canola—and had them tested for genetic sequences originating in transgenic crops. In 18 varieties (six of each crop), we looked for evidence of both regulatory sequences such as promoters, which control gene

expression, and genes, which confer herbicide resistance or insect resistance (Bt), from engineered varieties. We found pervasive, low-level contamination from transgenically derived sequences in the seeds of traditional varieties of all three crops.

Although we expected to detect some contamination, we were surprised to find transgenic sequences in most of the varieties tested. The varieties we tested were selected to represent a substantial portion of the 2002 seed supply for the traditional varieties of the three crops. That is, the 18 varieties we selected were marketed by major seed companies to farmers in the two states planting the most acres of corn and soybeans and the one state planting the most canola acres in the United States. Therefore, we tentatively conclude that seed contamination in those three crops is not limited to pockets of the seed supply, but is pervasive.

Although they are preliminary, the results of this study suggest the existence of an easy path for the movement of transgenes into the seed supply—one impeded little by current regulations or the standard confinement procedures in commodity crop seed production.

Chapter 3

Implications

Our pilot study suggests that the commercial seed stocks of non-engineered (traditional) commodity corn, soybean, and canola varieties are pervasively contaminated with low levels of sequences originating in genetically engineered varieties. The genes and genetic sequences we detected came from popular transgenic varieties currently allowed on the market in the United States.

Although the study sheds little light on how the contamination occurred, there is no reason to assume that the traits detected in this study were the only engineered traits moving into the traditional seed supply. We would not be surprised if further examination revealed additional traits contaminating a greater number of crop varieties. Until we know otherwise, it is minimally prudent to assume that *any* transgenes or transgenically derived sequences being produced and field tested in the United States could move into the seed supply of corn, soybeans, canola, or any other crops with engineered varieties. The vulnerability of the seed production system to contamination is due primarily to its design and standard operating procedures. Contamination is likely to continue unless that system is changed.

Assuming this report's conclusions are borne out by further study, its implications are broad. Seeds are fundamental to agriculture and the food supply, and continued seed contamination can have a potential impact in a number of arenas. We briefly address nine of these below: pharmaceutical and industrial crops, food safety, the environment, trade, organic food production, intellectual property, the food system, the agriculture of developing countries, and seed repositories. In Chapter 4, we present our conclusions and recommendations.

AREAS OF CONCERN

1. Pharmaceutical and industrial crops

The possibility of seed contamination for food crops heightens concerns about pharmaceutical and industrial crops.

Will drug-producing crops end up contaminating our seed and food supplies? Our results suggest reasons for concern. In the near term, this may be the most important implication of our findings.

Agricultural biotechnology is entering a new age. No longer are researchers concentrating only on inserting genes that result in plants with traits like herbicide and insect resistance that make crops cheaper or easier for farmers to grow. Now they are inserting genes to create plants that produce drugs and industrial chemicals—in essence turning the crops into biological factories. The developers of the new pharmaceutical-producing "pharm" crops especially promise compelling benefits: new drugs that would otherwise be unavailable, and decreased production costs leading to lower consumer drug prices.[49]

A wide variety of genes has been engineered into plants for pharmaceutical and industrial purposes. For more information, see the box,

49 Whether the technology can deliver on these promises remains uncertain. Production costs, for example, are just one factor in consumer drug prices, and drug companies often use patents on popular products to charge high prices unrelated to the costs of production and testing.

What kinds of substances are being engineered into pharm and industrial crops?

The following is a list (gleaned from public sources including industry websites) of experimental pharmaceutical and industrial substances that have been produced in engineered crops. Many of them are bioactive and/or toxic. Currently, no drugs produced in genetically engineered plants are on the market.

Pharmaceuticals or drugs: Proteins for healing wounds and treating conditions such as anemia, liver cirrhosis, and cystic fibrosis; anticoagulants; blood substitutes; hormones; and enzymes to treat Fabry's and Gaucher's diseases.

Antibodies: Substances that home in on disease-causing molecules with great specificity. Examples include antibodies to fight cancer and tooth decay.

Vaccines: Substances to be injected or given orally to humans and animals to confer immunity to diseases, including non-Hodgkin's lymphoma, rabies, cholera, piglet diarrhea, and foot-and-mouth disease. So-called "edible" vaccines are fruits and vegetables engineered to contain vaccines that will be delivered by ingestion. Currently being developed to fight diseases such as hepatitis B, measles, and polio, as well as various types of viral diarrhea, edible vaccines were originally envisioned in whole foods such as tomatoes that can be eaten raw, but dosing and quality control considerations have led most developers to consider at least minimal processing of foods and batch production.

Industrial chemicals: Compounds used in the manufacture of products such as paper, plastics, personal care items, and laundry detergents. Examples are trypsin and laccase.

Research chemicals: Substances used in investigative and diagnostic laboratories. Examples include avidin and beta-glucuronidase.

SOURCES: Pew Initiative on Food and Biotechnology (PIFB). 2001. Harvest on the Horizon: Future Uses of Agricultural Biotechnology. Washington, DC: PIFB, pp. 53-63 and references therein; Union of Concerned Scientists (UCS). 2003. Pharm and Industrial Crops: the Next Wave of Agricultural Biotechnology. Washington, DC: UCS, pp. 3-4 and references therein, on the UCS website at *http://www.ucsusa.org/publication.cfm?publicationID=538*, accessed June 19, 2003.

"What kinds of substances are being engineered into pharm and industrial crops?" Many crops containing these genes have been tested in the open environment. Corn is the crop most widely tested for use as a pharm crop, but other food and feed crops including rice, potatoes, soybeans, tomatoes, and canola are also being used. Appendix B includes a list of transgenes from USDA field test records, among which are a number of transgenes intended for pharmaceutical use. Many other pharm crop transgenes have been tested but their identities are withheld from the public as confidential business information.

The production of drugs and industrial chemicals in corn and other food crops presents obvious risks.[50] If genes find their way from pharm crops to ordinary corn, they or their products could wind up in drug-laced corn flakes. In addition, crops that unintentionally contain drugs or plastics could also prove harmful to domestic animals that eat contaminated feed; to deer, mice, birds, and other wildlife that feed in pharm crop fields; or to organisms living in the soil.

The prospect of pharmaceutical genes contaminating the seeds we depend on for our food supply is genuinely troubling. If seeds are contaminated

50 Union of Concerned Scientists (UCS). 2003. Pharm and Industrial Crops: the Next Wave of Agricultural Biotechnology. Washington, DC: UCS, pp. 9-11 and references therein, on the UCS website at *http://www.ucsusa.org/publication.cfm?publicationid=538*, accessed on June 19, 2003.

with genes for drugs, farmers will unknowingly plant and harvest what could be very dangerous crops. The fact that many pharm crops will be planted on small acreage does not assuage the concern.[51] The StarLink incident described in Chapter 1 involved crops planted on less than 0.5 percent of U.S. corn acreage, yet the product ended up contaminating grain throughout the food system. Also affected were the seed stocks of at least 63 small and medium-sized seed companies—more than one-fifth of those contacted by the USDA in the course of the department's seed buyback.[52] StarLink genes may still contaminate the seed supply.

The likelihood that seeds would become contaminated with genes from pharm crops is difficult to assess. It will depend on how the seed contamination occurs (by physical mixing or outcrossing) and a number of other factors, such as whether fields intended for seed production or seed increase for food and feed crops are located close to areas where pharm crops are grown. More study is needed to understand how often seeds are contaminated and where in the seed production process contamination occurs. At this point, we do not have the information to be assured that pharmaceutical genes have not already moved into our food system.

Pharm and industrial crops, for the most part, remain in the early phases of development. At this point, we should still be able to control the risks of this technology by imposing a strong new regulatory system. Now that we recognize that seeds could become contaminated with pharm or industrial products during the field testing phase and that these genes could make their way into commercial agricultural production, we need to ensure that the seed supply for food crops is explicitly protected in the development of such regulations.

2. Food safety

The prospect of pervasive seed contamination raises food safety concerns for the future, although the particular genes detected in this study do not set off alarms.

There is no reason to believe that genetic sequences originating in transgenic crops *per se* render food unhealthful. Only if the genes or their products cause problems on ingestion is there a food safety hazard, a determination that needs to be made on a case-by-case basis.

The transgenically derived sequences detected in seeds of traditional varieties in this study include both regulatory sequences (e.g., promoters) and genes conferring the traits of interest from the two most popular kinds of transgenic products on the market today. These varieties have passed through the government oversight system for food safety, although only the Bt crops were formally approved for food use by a federal agency—the Environmental Protection Agency (EPA).[53]

Within the limits of that system, we have no evidence that these transgenically derived sequences are not safe and we do not believe

51 Field trials conducted before commercialization usually start with very small plots (less than 1 acre to 10 acres) but can increase dramatically as products get closer to market. At commercialization, some of these products—therapeutic vaccines and certain research chemicals, for example—will likely require only tens of acres to meet the specific demands of those particular markets. Other products, however, will necessitate much larger plantings, ranging up to hundreds of thousands of acres.

52 U.S. Department Agriculture (USDA). 2001. USDA purchases Cry9C affected corn seed from seed companies. USDA News Release, June 15, on the USDA website at *http://www.usda.gov/news/releases/2001/06/0101.htm*, accessed on November 14, 2003.

53 The EPA formally approves crops that are engineered to produce plant-incorporated protectants (PIPs) such as the Bt toxin. The agency does not regulate herbicide-resistant crops as PIPs. The Food and Drug Administration (FDA) does not formally approve genetically engineered crops; it merely encourages developers to engage in a voluntary consultation process after which the agency affirms that it has no questions about a biotechnology company's determination of product safety. (FDA. 1992. Statement of policy: foods derived from new plant varieties. *Federal Register* 57:22984-23005.)

their detection in this study raises food safety alarms. Given the lack of monitoring systems in the United States, lack of reported incidents is not strong evidence of lack of effect, but food ingredients made from these products have been consumed for several years without the emergence of overt problems connected to their origin via genetic engineering.

We do, however, have reservations about the safety of genetically modified food. Our concerns are related less to known problems with the products currently on the market than the lack of scientific rigor in the system evaluating their safety. We have long stressed the need for a mandatory system that would provide a government-backed finding of safety, and have urged the government to undertake or support new basic research to evaluate the potential hazards of genetically modified food (e.g., in the area of allergenicity).[54] As it stands now, the Food and Drug Administration (FDA) has little power to compel companies to submit food safety data and does not carry out independent, scientifically rigorous reviews of new transgenic food products.[55]

We do, however, have reservations about the safety of genetically modified food. Our concerns are related less to known problems with the products currently on the market than the lack of scientific rigor in the system evaluating their safety.

A new, stronger system would inspire a higher degree of public confidence in the safety of engineered foods, particularly those products that will be brought to market in the future. The system should be based on more rigorous science and include more tests for unexpected effects, as recommended recently by the Codex Alimentarius Commission, an international body that sets food safety standards under the auspices of the Food and Agriculture Organization and the World Health Organization.[56] The U.S. National Academy of Sciences recently conducted a study on the hazards and unintended impact of engineered food on human health, and is preparing a report expected to contain recommendations for improving the food safety assessment process.[57]

While most novel gene products will probably prove safe to consume as food and feed, such products are not inherently safe. For gene products that turn out to be harmful, the general concern in the seed contamination context is that the products will make their way into non-engineered seed varieties and be perpetuated in those crops by successive breeding cycles. The new products might not be readily identified as harmful because

54 Allergenicity is one of the major challenges in the evaluation of a genetically modified food's safety. Scientists currently have only limited ability to predict the allergenicity of a particular protein on the basis of its biophysical characteristics. As a result, the protocols used to screen for allergens on the basis of such characteristics are necessarily imperfect. The StarLink variety of Bt corn was denied approval for food uses because its Bt toxin failed screens for digestibility and heat stability. StarLink raises the question of whether other Bt toxins that passed the screens might nevertheless be allergens. It is difficult to resolve this question without a better understanding of food allergenicity. The failure to identify and remedy such a critical research need is a major flaw in the U.S. system overseeing genetically engineered food.

55 Gurian-Sherman, D. 2003. Holes in the Biotech Safety Net: FDA Policy Does Not Assure the Safety of Genetically Engineered Foods. Washington, DC: Center for Science in the Public Interest (CSPI), on the CSPI website at *http://cspinet.org/new/pdf/fda_report__final.pdf*, accessed on February 5, 2003.

56 Haslberger, A.G. 2003. Codex guidelines for GM foods include the analysis of unintended effects. *Nature Biotechnology* 21:739-741 (July).

57 National Academy of Sciences (NAS). 2003. Project title: unintended health effects of genetically engineered foods. Project Identification Number: BBXX-K-00-02-A. On the NAS website at *http://www4.nationalacademies.org/cp.nsf/projects+_by+_pin/bbxx-k-00-02-a?opendocument*, accessed on December 18, 2003.

they would occur sporadically in products not recognized as genetically engineered.

The degree of concern about potential contaminants of food and feed crops varies with their regulatory status and intended use. Transgenic products that have undergone government scrutiny for use in or as food and feed (e.g., for herbicide and insect resistance) tend to raise the least concern. These products were developed for human and animal consumption and have at least been evaluated at some level and screened for obvious problems.

Transgenic products that have not undergone food safety review but have been and are still being field tested raise more concern. Under the U.S. regulatory system, agencies do not analyze genetically modified crops for food safety until after they have undergone years of field testing. This means that transgenic crops are potentially available to contaminate the seed supply long before a decision has been made about their safety. Examples of engineered crops that have been field tested but not evaluated for food safety include rice resistant to fungal diseases and corn with modified oils, starches, and proteins.[58] Although not necessarily harmful, transgenic crop varieties that have not been scrutinized are of greater concern than scrutinized products because they have undergone no screen to remove dangerous transgenes.

Finally, gene products that are not intended for use in food raise the highest level of concern. They are unlikely to be reviewed for food safety at all, and many, such as pharm and industrial crops, are likely to produce bioactive and toxic compounds.

Ad hoc accumulation of several novel genes raises food safety concerns. In seed production systems that allow new genes to move into seeds via cross-pollination, every season offers new opportunities for the introduction of new traits. Single plants could accumulate and propagate several different novel traits over time, especially if they offer selective advantages. For example, in the short time that herbicide-resistant canola has been grown in Canada, genes for resistance to three different herbicides have accumulated in individual canola plants—whose offspring show up as weeds in fields planted with canola and other crops. Two of the resistance traits originated in engineered canola varieties and one came from a traditionally bred variety.[59]

Whatever food safety dangers may accompany the presence of single novel genes, combinations of genes raise new concerns. The combinations of traits would not likely have been reviewed by agencies for food safety and may present synergistic or otherwise unpredictable effects. Accumulation (or the natural stacking) of traits is most likely to occur in crops whose seeds are routinely saved and planted. Parental lines of hybrid crops or true-breeding crops such as canola or soybeans fit in this category.

3. The environment

The additional risk posed by a transgene contaminating traditional varieties of a crop is likely to be small where the transgene is already present in widely planted commercial varieties of the same crop. Seed contamination, however, offers new routes by which transgenes might make their way surreptitiously to new environments—with unknown effects.

Just as with food safety, the presence of engineered traits in the supply of traditional seeds is not *necessarily* a problem from an environmental

58 Information Systems for Biotechnology (ISB). 2003. Field Test Releases in the U.S. Blacksburg, VA: Virginia Polytechnic Institute and State University. On the ISB website at *http://www.isb.vt.edu/cfdocs/fieldtests1.cfm*, accessed on October 14, 2003.

59 Hall, L., K. Topinka, J. Huffman, L. Davis, and A. Good. 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Science* 48:688-694.

perspective. Nothing about genetic engineering suggests that in and of itself, gene products derived from transgenic crops constitute an environmental threat or that engineered sequences inevitably render non-engineered plants dangerous to the environment.

But, again, neither are all such crops inherently safe; some do present environmental risks. The nature and degree of these risks depend on the traits added, the plants to which they are added, and the environment within which the plants are situated. Environmental risks are complex in nature and highly context-dependent.

We address the risk issue here within the framework of our earlier report, *The Ecological Risks of Engineered Crops*.[60] That book organized the risks of genetically engineered crops around the notion of weeds—a generic term for plants unwanted by humans, whether in agricultural or nonagricultural settings. In this context, weeds include not only those plants that compete with crops but also those plants that degrade environments of value to humans. Thus, purple loosestrife that decreases the usability of a pond ecosystem by ducks, duckweed that clogs water channels, and kudzu that kills trees are all weeds.

The main environmental risk of genetically engineered crops is that they would become weeds or transfer traits to wild relatives that would become weeds. Whether crops become or give rise to weeds depends on the genes they carry and, importantly, where they are grown. Crops cannot contribute genes to wild and weedy relatives if none exist nearby.

One question here is what additional risk to the environment is posed by a transgene present as a contaminant in traditional varieties of a crop beyond the risks posed by the growth of commercial varieties containing the transgene already permitted in commerce. In general, as long as the level of contamination remains low, where the transgenes at issue have been allowed on the market and the varieties containing them are widely adopted, the increased exposure due to the contaminants in the seed supply is unlikely to substantially increase exposure to the transgenes or the overall risk. The increase in the levels of Bt toxin coming from contaminated corn seed, for example, will not add much to the overall pressure on the environment resulting from the stream of Bt toxins already in the environment due to commercial Bt products.[61]

In most cases, neither seed sellers nor farmers would be aware of the contaminant, which would undermine their ability to effectively manage for environmental risks.

On the other hand, seed contamination offers genes and gene products surreptitious paths to new environments. In most cases, neither seed sellers nor farmers would be aware of the contaminant, which would undermine their ability to effectively manage for environmental risks. The greatest risks would be associated with untested or disallowed genes, but even allowed genes might be a problem.

For example, transgenic salt-tolerant rice might be commercialized under conditions designed to keep the plants from invading coastal wetlands.

60 Rissler, J. and M. Mellon. 1996. *The Ecological Risks of Engineered Crops*. Cambridge, MA: MIT Press.

61 Contamination is also generally unlikely to reduce the performance of the crop. For example, a small amount of seed of a drought-tolerant variety planted along with seed of a non-drought-tolerant variety will not interfere with the field production of the non-tolerant variety.

If the transgenes for salt tolerance were to contaminate the traditional rice seed supply, however, their presence would not be known and no precautions taken. The contaminated rice seeds, finding their way to wetlands, could upset a delicate and important ecosystem. Furthermore, if the traits conferred a selective advantage, this would increase the prevalence of the transgenes in the new system.

It is also possible that plants from the transgenic contaminating seeds could breed with wild plants, transferring new traits into wild populations. The effects of such transfers depend on the traits, the receiving populations, and environmental pressures and stresses. But harmful effects are certainly possible.[62] The possibility that some of the large number of transgenes that have already been field tested in more than 40 food and feed crops may already be moving into wild plant populations is troubling.

In contrast to food and feed safety concerns, the relatively low level of contaminating transgenes found in any particular seed batch is not a limit on the amount of harm these transgenes can do in the environment. Considerations of ecological risk must take into account the ability of favorable environments to select for and increase the proportion of harmful transgenes in plant populations.

Contamination with transgenes from pharm and industrial crops raises environmental issues of special concern. These genes may be the sources of toxins that harm wildlife. In addition, toxin production is a common strategy by which plants protect themselves from predators, and pharm genes may provide selectable advantages in wild plant populations. If such transgenes are transferred from pharm crops to weedy relatives or used in crops that have tendencies to become weeds, they may enable crops to become weeds or make existing weeds more resilient and difficult to control.

For example, aprotinin, a cow protein that has human medical uses but is also an insect toxin, has been produced in engineered corn plants.[63] If aprotinin genes were to move from aprotinin-producing pharm crops into weedy relatives, the new genes might make the weeds hardier by enhancing their ability to withstand insect predation. The likelihood of pharm genes establishing themselves in weedy populations is enhanced where the pharm genes confer an advantageous trait such as insect resistance.

In contrast to food and feed safety concerns, the relatively low level of contaminating transgenes found in any particular seed batch is not a limit on the amount of harm these transgenes can do in the environment.

Seeds contaminated with Bt insect-resistance transgenes could also undermine the effectiveness of so-called resistance-management refuges. Refuges are non-engineered crops planted in the

62 Ellstrand, N.C., H.C. Prentice, and J.F. Hancock. 2002. Gene Flow and Introgression from Domesticated Plants into Their Wild Relatives. In *Horizontal Gene Transfer*, second edition, ed., M. Syvanen and C.I. Kado, 217-236. London: Academic Press.

63 Zhong, G.-Y., D. Peterson, D.E. Delaney, M. Bailey, D.R. Witcher, J.C. Register III, D. Bond, C.-P. Li, L. Marshall, E. Kulisek, D. Ritland, T. Meyer, E.E. Hood, and J.A. Howard. 1999. Commercial production of aprotinin in transgenic maize seeds. *Molecular Breeding* 5:345-356. A joint commercial research venture involving Pioneer Hi-Bred (a major seed company), Prodigene (a pharm crop company), and Eli Lilly (a major pharmaceutical company) has successfully engineered corn to synthesize aprotinin.

In general, seed contamination provides an avenue for release into the environment of genes and gene products that have not been evaluated or allowed in commerce and whose presence is unknown to farmers, regulators, or scientists. These may cause problems that are difficult to identify and remedy.

proximity of engineered Bt crops to slow the evolution of insect pests resistant to the Bt toxin. In theory, refuges work by allowing populations of susceptible pests to survive and mate with the relatively rare resistant pests. If the refuges are contaminated with Bt-producing plants, they would likely kill some susceptible pests, thereby aiding the emergence of Bt-resistant insects. Whether the presence of Bt transgenes in traditional varieties of crops would affect the efficacy of refuges would depend on the levels of contamination. Low levels of contamination would be unlikely to have much effect.

In general, seed contamination provides an avenue for release into the environment of genes and gene products that have not been evaluated or allowed in commerce and whose presence is unknown to farmers, regulators, or scientists. These may cause problems that are difficult to identify and remedy.

Finally, just as we noted about food safety, our concerns about the environmental risks of engineered crops are exacerbated by the federal government's weak regulatory oversight, its lack of scientific rigor in risk assessments, and its failure to adequately address unintended consequences. The National Academy of Sciences, in recent reports, has criticized both the USDA and the EPA—the two agencies charged with environmental oversight—for failing to develop strong, rigorous regulatory programs.[64]

4. Trade

Seed contamination exacerbates the difficulty of providing non-engineered products to demanding import customers.

Corn and soybeans are major export crops. The United States produces far more of these crops than its own economy can absorb, so it sells aggressively to the rest of the world. While engineered crops are popular among U.S., Argentinean, and Canadian farmers,[65] they are highly controversial in other parts of the world, most importantly among some of our major trading partners such as the European Union, Japan, and South Korea.[66]

Resistance in these and other countries has led to a complex set of serious problems for U.S. exporters,[67] most of which are the result of the

64 National Research Council. 2000. *Genetically Modified Pest-Protected Plants: Science and Regulation*. Washington, DC: National Academy Press; National Research Council. 2002. *Environmental Effects of Transgenic Plants: the Scope and Adequacy of Regulation*. Washington, DC: National Academy Press.

65 International Service for the Acquisition of Agri-biotech Applications (ISAAA). 2003. Global Status of GM Crops: Global Area of GM Crops in 2002. On the ISAAA website at *http://www.isaaa.org/kc/bin/gstats/briefs.htm*, accessed on June 17, 2003. The United States planted two-thirds of the global acreage of genetically engineered crops in 2002. Four countries accounted for 99 percent of the total: United States (66 percent), Argentina (23 percent), Canada (6 percent), China (4 percent).

66 The causes of this resistance are many and complicated. Some resistance stems from consumer concerns and some from desires to protect markets. In addition, genetic engineering has often been presented as an "our way or the highway" proposition, stirring up resentment in parts of the world concerned about looming U.S. hegemony. Finally, there are legal implications to contamination with products that have not been approved in other countries.

67 For more information on trade implications of genetically engineered contaminants, see Taylor, M.R. and J.S. Tick. 2003. Post Market Oversight of Biotech Foods: Is the System Prepared? Washington, DC: Pew Initiative on Food and Biotechnology, pp. 58-84.

United States' failure to supply non-engineered bulk products sufficiently free of transgenically derived sequences. This inability is somewhat surprising, considering that the United States, like any good marketer in a competitive industry, should want to satisfy customer demands and capture market share.

The United States grows substantial quantities of non-engineered and organic products that face no customer resistance anywhere in the world and which, in many cases, even command premium prices. But much of the non-engineered grain and oilseed is contaminated with varying levels of genetic sequences derived from engineered varieties. This would not matter if U.S. export customers tolerated contamination with engineered sequences to the same degree they tolerate contamination with other varieties or even other crops, but that is not the case. Many customers want grain or oilseed free of transgenic sequences, especially genes or gene products that have not been approved in their countries.[68] Meeting this demand has proved a difficult challenge.[69]

Most of the contamination of bulk grain and oilseed products is the result of physical mixing that occurs routinely within the infrastructure of trucks, ships, and grain elevators that moves commodity crops to market. In addition, the outcrossing of pollen from engineered plants into neighboring fields is unavoidable. The existing commodity infrastructure was never intended to transport different segregated streams of grain and oilseed from farms to food and feed processors. As long as the United States grows substantial acreages of engineered crops and does not alter its commodity infrastructure, it will not be able to readily provide uncontaminated commodity grain or oilseed product.

Seed contamination exacerbates the difficulty of keeping engineered genetic sequences out of non-engineered grain and oilseed. Even if growers seeking to export highly pure non-engineered commodity crops could start with pure seed, unreviewed or unwanted transgenic sequences could move into their products via mixing or outcrossing. But, when farmers start with contaminated seed, even the most innovative systems for moving segregated products to market are doomed. Such systems represent new market opportunities and are currently the focus of substantial investments.

It should also be noted that customer preferences are moving targets. If international customers grow more accepting of engineered grain and oilseed, the intermixing inevitable within the current commodity system would cause fewer problems for U.S. exports, and the importance of seed contamination as a contributor to trade problems would be diminished.

Global resistance to genetic engineering, on the other hand, could continue to stiffen and perhaps reach a point where the United States would have to retool parts of its commodity grain and oilseed infrastructure to enable the segregation of uncontaminated non-engineered products. As discussed earlier, the trend in U.S. agriculture is toward identity-preserved systems.[70] In this scenario, the value of pure non-engineered seed to U.S. exports would increase.

68 Demetrakakes, P. 2000. Processors are trying to gauge the meaning of the backlash against genetically modified crops. *Food Processing Magazine* (March 1). On the *Food Processing Magazine* website at *http://www.foodprocessing.com/web_first/fp.nsf/articleid/meat-4l8nvb*, accessed on November 14, 2003; McMillan, D. 1999. We must provide what customers want. *Western Producer* (September 2). On the *Western Producer* website at *http://www.producer.com/articles/19990902/market_quotas/opmcmillan.html*, accessed on November 14, 2003. Growers can have similar problems in the domestic U.S. market with demanding customers such as baby food manufacturers, many of which also prefer foods free of transgenically derived sequences.

69 *The Non-GMO Source*. 2001. Export buyers concerned about US ability to provide non-GMO. Volume 1, Number 3, pp. 1-3 (June). The inability to supply the products customers demand has lost the United States important markets, most notably in the European Union, but also in Japan.

70 Strayer, D. 2002. *Identity-Preserved Systems: A Reference Handbook*. Boca Raton, FL: CRC Press.

5. Organic food production

The contamination of traditional seed supplies undermines the future of organic agriculture.

Food products that bear the federal organic seal and label have met the U.S. government's standards for the growing and handling of organic food. The core organic standards restrict the use of synthetic pesticides and prohibit the use of irradiation, municipal sludge, and engineered seeds and other engineered inputs in food production. The combination of comprehensive and stringent standards and management systems that enable farmers to meet these standards comprises a holistic approach to food production that works in concert with the environment.

Food that meets organic standards generally commands a premium price in the marketplace. In fact, organic food has sufficient appeal that it is one of the few sectors of U.S. agriculture that is maintaining long-term, double-digit annual growth rates.[71] U.S. certified organic cropland and pasture more than doubled between 1992 and 2001, from fewer than one million acres in 1992 to 2.3 million acres in 2001.[72] Because the organic market offers a value-added product especially important for small and medium-sized farms, the potential loss of this market is of growing importance to U.S. agriculture.

Many organic buyers, processors, and consumers, like many U.S. export customers, are demanding a product free of transgenically derived sequences.[73] To the extent that U.S. organic farmers cannot meet that demand, consumers will go elsewhere or perhaps refuse to pay premium prices. The U.S. government, which touts its organic label as the equivalent of a label indicating the absence of genetically engineered sequences, also has an interest in helping organic growers meet the demand.

As discussed above, organic farmers are struggling to find uncontaminated seed. If they cannot purchase seed free of transgenically derived sequences or control post-planting outcrossing—neither of which is completely within their control—they will be unable to meet their own or larger societal demands for non-engineered food. Although it is only one part of the solution, the availability of seed free of engineered substances is essential to meeting consumer demand and preserving an increasingly important sector of U.S. agriculture.

Although it is only one part of the solution, the availability of seed free of engineered substances is essential to meeting consumer demand and preserving an increasingly important sector of U.S. agriculture.

71 Dimitri, C. and C. Greene. 2002. Recent Growth Patterns in the U.S. Organic Foods Market. USDA Economic Research Service (ERS), Market and Trade Economics Division and Resource Economics Division, Agriculture Information Bulletin Number 777 (September). On the USDA ERS website at *http://www.ers.usda.gov/publications/aib777/aib777.pdf*, accessed on December 15, 2003.

72 Dimitri, C. and C. Greene. 2002; Greene, C. and C. Dimitri. 2003. Organic agriculture: gaining ground. *Amber Waves: the Economics of Food, Farming, Natural Resources, and Rural America* (February). On the USDA ERS website at *http://www.ers.usda.gov/amberwaves/feb03/findings/organicagriculture.htm*, accessed on December 15, 2003.

73 Yates, S. 1999. Exported corn chips tainted with GMOs. *Natural Foods Merchandiser* (April). On the New Hope Natural Media website at *http://exchange.healthwell.com/nfm-online/nfm_backs/apr_99/cornchips.cfm*, accessed on May 10, 2003; *The Non-GMO Source*. 2002. Organic farmers report increasing problems with GMO contamination. Volume 2, Number 12, pp. 1-2 (December). Although organic standards do not strictly require a product free of genetic engineering, organic farmers are in a bind because they cannot control the contamination caused by outcrossing originating in their neighbors' fields. They can and have been severely penalized in the marketplace when, through no fault of their own, their harvested products contained traits they did not plant.

6. Intellectual property

Contamination of non-engineered seeds subjects farmers who have never purchased engineered seeds to the intellectual property laws.

The pervasive contamination of seeds may also have patent implications for farmers who inadvertently plant and harvest seed containing transgenically derived sequences. Under U.S. intellectual property laws, genes, gene products, and engineered crops are now considered patentable subject matter—just like windshield wipers or clocks.[74] Where patents apply, it is illegal for others to make, use, or sell the invention during the term of the patent without permission from the patent holder. To do so could subject the infringer to lawsuits and stiff penalties.

An important feature of the patent law is that infringement does not require intent. Farmers who use genes or seeds patented by others can be sued even if they did not know they were using the invention. While the law is murky, pervasive seed contamination would appear to put farmers at risk of unknowingly infringing the patents held by biotechnology companies. The threat of patent holders pursuing infringement claims against farmers who inadvertently purchased contaminated seed seems counterintuitive, but it is not impossible. Monsanto, for example, has not been shy about bringing suits against farmers for patent infringement, despite having provoked widespread anger and resentment in rural America.[75]

7. The food system

Seeds contaminated with transgenically derived sequences add a new source of potential food system disruption to the already difficult problems posed by bulk contamination.

The presence of unapproved genes and gene products would of course play havoc with the food system if the traits they confer proved to be harmful, but this could be the case even if they were not harmful. In general, food handlers and processors are not allowed to sell food considered to be adulterated under the provisions of the Federal Food, Drug and Cosmetic Act (FFDCA). Food may be considered adulterated for many reasons, including the presence of either pesticidal substances for which the government has not set a tolerance[76] or unapproved food additives.[77] Products of genetic engineering could fall in either category.

The StarLink episode discussed in Chapter 1 illustrates just how disruptive the presence of an unapproved pesticidal product in the grain and food system can be. The EPA had approved StarLink (a variety of corn engineered to contain a pesticidal Bt toxin) for animal feed but not human food in 1997. The announcement that StarLink corn had been found in taco shells in 2000 set into motion widespread product recalls. Without a tolerance set by the EPA, the presence of the Bt pesticide rendered food adulterated under the FFDCA and therefore illegal.[78] The

74 Diamond v. Chakrabarty, 447 U.S. 303 (1980).

75 Monsanto still suing Nelsons, other growers. Cropchoice.com (May 21). On the Cropchoice.com website at *http://www.cropchoice.com/leadstry.asp?recid=326*, accessed on June 23, 2003.

76 21 U.S.C. 342(a)(2), 346.

77 21 U.S.C. 342(a)(2), 348.

78 21 U.S.C. 346a(a)(1).

recalls set off an expensive chain of events as grain sellers and food handlers had to test and divert contaminated lots of grain.[79]

The sources of transgenes that may move into the food supply and trigger similar disruptive events include food crops grown for non-food purposes (for example, corn used as a pharm crop) and engineered crop varieties in the early stages of development prior to commercialization. Transgenes from these sources might be physically mixed with or outcross into food crops destined for the food system, where they could cause widespread disruption including recalls and lawsuits if discovered.

Seed contamination would exacerbate this problem by making it even more difficult for growers and food companies to know the exact composition of the products they buy and sell.

8. Agriculture of developing countries

Contamination of non-engineered seed in the United States may increase the unpredictability of agriculture in developing countries and may lead to or exacerbate contamination of traditional crop varieties, landraces, and wild progenitors in centers of diversity.

There are two ways that seeds contaminated with engineered sequences could make their way to developing countries: as seeds for planting or as bulk products, which are made up of viable seeds. In developing countries, it is highly likely that seeds purchased as commodity products will be planted by farmers as seeds.

Unsuspecting purchasers of potentially contaminated traditional seed in developing countries will take no precautions to prevent the flow of transgenes into nearby crops and wild and weedy relatives via outcrossing. Since U.S. seeds could be contaminated with many kinds of genes, the consequences of gene movement are difficult to predict. Transgenes that confer fitness benefits on plants can become fixed in plant populations and increase in frequency in successive generations.[80] Thus, seed contamination could become a conduit for new genes—some of which may be harmful to human health or the environment—into wild and weedy plants.

Unsuspecting purchasers of potentially contaminated traditional seed in developing countries will take no precautions to prevent the flow of transgenes into nearby crops and wild and weedy relatives via outcrossing.

In general, the most unsettling aspect of seed contamination for producers in the developing world is that there is no way to evaluate, monitor, or avoid such movements because they would occur surreptitiously. Where transgenes move into other varieties or landraces,[81] they could lead to unpleasant—and expensive—surprises. For example, if herbicide-resistance genes move into crop varieties, farmers may find that costly

79 The StarLink-related losses for food recalls, lost sales, payments to farmers and grain elevators, and seed buybacks amounted to hundreds of millions of dollars. The USDA ended up bailing out seed companies involved in the effort to contain the contaminants. Demand for U.S. corn abroad plummeted. (Gillis, J. 2003. Little oversight of altered crops. *Washington Post* [April 25]; Howie, M. 2003. Non-StarLink growers reach class action settlement. *Feedstuffs* [February 24], p. 23; Lambrecht, B. 2001. *Dinner at the New Gene Café.* New York, NY: St. Martin's Press, pp. 51-55; Taylor, M.R. and J.S. Tick. 2003, pp. 90-105.)

80 Ellstrand, N.C. et al. 2002. Where gene flow is recurrent, even traits with detrimental effects can persist in a plant population.

81 Landraces are plants selected by traditional farmers from wild populations.

herbicides do not work. Or, if seeds of traditional corn varieties are contaminated with the Bt toxin gene, farmers may find that the crop unexpectedly kills beneficial insects.

The impact of contaminated seed must be considered against the backdrop of genetically engineered varieties that enter a country legally and fully disclosed as a bulk commodity product. It is likely, for example, that Bt transgenes in Bt crop varieties diverted for use as seed would flow through pollen into neighboring crop varieties, landraces, and wild relatives. For popular transgenes such as Bt, the seed diverted from transgenic varieties of commodity crops is likely to be a greater source of novel genes in developing countries than those same transgenes occasionally contaminating seeds of traditional varieties.

The 2001 discovery that landraces of corn in Mexico are contaminated with genetic sequences that originated in engineered corn varieties from the United States underscores the difficulty of confining transgenes used in agriculture.[82] Subsequent studies have confirmed and extended those findings.[83] It is not clear how the genes traveled to Mexico—whether seeds unapproved in Mexico were sold on the black market or bulk products imported from the United States[84] were diverted and used as seed. The Mexican government is attempting to assess the causes and consequences of this finding.[85]

The unexpectedly rapid dispersal of transgenes to Mexico only a few years after their first commercial use in the United States deserves immediate attention from the scientific community because Mexico is the center of diversity[86] for corn, one of the world's most important food crops. Teosinte, the crop's wild progenitor, can be found growing in Mexican cornfields, and whatever novel genes are found in Mexican landraces are also likely to be transferred into teosinte plants via pollen. While it is impossible with our current level of knowledge to assess the impact of novel genes on teosinte populations, the potential contamination of such important populations of wild plants points to the need for additional research.[87]

The ongoing situation in Mexico highlights the ease with which novel genes and traits can move through agricultural varieties into wild plant populations, including the vital populations that are the centers of diversity for important crops.

9. Seed repositories

If transgenes continue to move into the commercial seed supply of traditional crop varieties, seed repositories may also become pervasively contaminated with a variety of novel genes.

Ongoing contamination of the commercial seed supply could gradually undermine the quality of our communal genetic storehouse

82 Quist, D. and I. Chapela. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414:541-543 (November 29).

83 Alvarez-Morales, A. 2002. Transgenes in maize landraces in Oaxaca: Official report on the extent and implications. Abstract of presentation at the 7th International Symposium on the Biosafety of Genetically Modified Organisms, Beijing. On the 7th International Symposium website at *http://www.worldbiosafety.net/title%20paper.htm*, accessed on August 14, 2003.

84 Weiner, T. 2002. In corn's cradle, U.S. imports bury family farms. *New York Times* (February 26). Mexico imports about one-fourth of its corn from the United States.

85 Alvarez-Morales, A. 2002.

86 Centers of diversity are regions around the world that harbor populations of free-living relatives of crops. These populations serve as reservoirs of genes that can be moved into crops by traditional breeders.

87 Sánchez-González, J. 2002. Concerns about the effect of transgene introgression in maize landraces and teosinte. Abstract of presentation at the 7th International Symposium on the Biosafety of Genetically Modified Organisms, Beijing. On the 7th International Symposium website at *http://www.worldbiosafety.net/title%20paper.htm*, accessed on August 14, 2003.

for agricultural crops. Nothing is more fundamental to the future of our agriculture and food system than a continued supply of safe, high-quality seed.

The prowess of genetic engineers notwithstanding, seeds cannot be made from scratch. They must be produced generation after generation through highly complex, natural biological processes. The value to food and fiber production embodied in the seeds entrusted to our generation cannot be overstated.

Plant genetic storehouses are maintained through dynamic processes that involve saving, selecting, and storing seeds.[88] A number of groups and institutions are involved in this process. First, as we discussed above, commercial seed companies develop and sell seeds for crop varieties destined for fields or home gardens, in some cases in cooperation with farmers, gardeners, and scientists.[89]

Public-sector plant breeders also develop new varieties, although their role has diminished over the last several decades.[90] Despite the overall decline in university and other public-sector breeding programs, there are new projects under way in land-grant university systems, including the Public Seed Initiative, a joint venture among Cornell University, the USDA, and two organic farming groups.[91]

Farmers also continue to be active in seed selection and preservation. In fact, most of the world's farmers do not have access to commercial seed products and save seeds every season for planting the next season.

In addition to seed stores that are actively managed by companies, scientists, and farmers, some seeds are gathered and kept in repositories called seed banks. Some of the most important seed banks house collections managed by international organizations such as the Consultative Group on International Agricultural Research.[92] These seeds contain genes for valuable traits and combinations of traits that have been selected in a process spanning countless generations. Seed banks are not static collections; the seeds are often removed and planted, and their progeny returned to the seed bank.

Although motley and uneven in its importance to different farmers and gardeners, the sprawling network of seed repositories is vital to the quality and resilience of our food supply. Its importance suggests that we should be highly conservative in our judgment about potential threats to its integrity.

Contamination of seed repositories by transgenically derived sequences is not theoretical. The Charles M. Rick Tomato Genetics Resource Center at the University of California, Davis, recently reported that its seed stock had become contaminated with transgenes originating in a tomato variety engineered to alter processing characteristics. Seed bank officials moved immediately to recall contaminated seed samples that had been sent to researchers in the United States and 14 other countries since 1996.[93]

The magnitude of the threat posed by transgenically derived sequences is not known at this

88 Periodic planting and seed harvesting to replenish stores and increase viability provides opportunities for contamination.

89 Wheat breeding is a good example of companies, farmers, and university scientists working together.

90 Knight, J. 2003. A dying breed. *Nature* 421:568-570 (February 6).

91 See the Public Seed Initiative website at *http://www.plbr.cornell.edu/psi*.

92 For more detail, see the Consultative Group on International Agricultural Research website at *http://www.cgiar.org/research/res_genebanks.html.*

93 University of California (UC), Davis. 2003. Tomato seed from seed bank found to be genetically modified. Press release, UC Davis News and Information, December 18, on the UC Davis website at *http://www.news.ucdavis.edu/search/printable_news.lasso?id=6833&table=news*, accessed on December 19, 2003.

point. As we discussed above, engineered traits *per se* are not necessarily a problem from either an environmental or human health standpoint. Agricultural scientists do not knowingly create harmful varieties (other than, perhaps, for pharm and industrial crops.) Eventually, if engineered varieties are used for a long period without ill effect, seeds from certain engineered varieties will likely be added to seed collections intentionally.

Our experience with transgenic crops to date seems encouraging, but it is limited to a few traits in a few commodity crops. Pharm and industrial crops and other new products[94] dramatically different from Bt and herbicide-resistant products are on the cusp of development, and these engineered crops will exhibit new traits and other features that may warrant a higher level of concern than the first generation of transgenic crops.

At this juncture, there remains the remote possibility that the current assurances of safety may be proven wrong—that interfering with natural genetic systems could be setting something seriously amiss. We may be violating rules we do not know exist, passing transgenic sequences into food crops that are in some way generally debilitating, but that we have not yet noticed. Such effects may be accumulating gradually or may need to reach some threshold to manifest themselves.

Until we gain a better understanding of genetic engineering, it is premature to allow transgenically derived DNA and transgenic seeds to creep unobserved into seed repositories.

94 For a discussion of potential new kinds of engineered crops, see Wolfenbarger, L., ed. 2002. Proceedings of a Workshop on Criteria for Field Testing of Plants with Engineered Regulatory, Metabolic and Signaling Pathways, June 3-4, 2002. Blacksburg, VA: Information Systems for Biotechnology (ISB), Virginia Polytechnic Institute and State University. On the ISB website at *http://www.isb.vt.edu/proceedings02/the_proceedings02.pdf*, accessed on August 14, 2003.

Chapter 4

Conclusions and Recommendations

CONCLUSIONS

The results of the pilot study presented in this report suggest that seeds of traditional varieties of corn, soybean, and canola sold to growers in the United States contain low levels of genetic sequences that originated in engineered crops. Because we tested seeds of varieties representative of a substantial portion of the 2002 traditional seed supply, we believe that contamination is not an isolated phenomenon but is endemic to the system.

Widespread contamination with genetically modified sequences suggests that production systems for seed sold in the United States are porous—that is, as currently designed and operated, these systems routinely allow contamination by other crop varieties, including engineered varieties. Unless these standard procedures are tightened, there is little reason to believe that the current level of contamination will decrease. Business-as-usual seed production ensures the perpetuation of contamination and a probable increase in the level and extent of contamination. This is a critical moment for the growers, traders, and food companies who are bearing the managerial costs of testing and segregation, and who will incur liability if incidents similar to StarLink occur again.

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In percentage terms, the reported levels of contamination are very low. Nevertheless, as illustrated in Chapter 2, such levels could result in tons of genetically engineered seed being planted each year intermixed with non-engineered seed.

Current concerns: food safety, environment, trade

Today, there is no reason to believe that low-level contamination of non-engineered food crops with genetic sequences from the two kinds of transgenic crops (Bt and herbicide-resistant) detected in the study represents a major threat to human health or the environment. The crops have undergone federal review, and while we agree with the National Academy of Sciences reports indicating that the U.S. regulatory system is not as rigorous as it should be, we do not believe this report justifies raising alarms about the currently unresolved food safety or environmental issues surrounding these crops. Since genetically engineered crops expressing these traits already represent a substantial portion of the grains and oilseeds produced in the United States, the additional presence of contaminants in non-engineered versions of those crops probably represents a marginal increase in dietary or environmental exposure.

Any assurances about food, feed, and environmental safety, of course, apply only to transgenes that can be detected through testing.

Unfortunately, for practical reasons, many transgenes—including those that have not survived the development process—cannot be detected with PCR-based tests. There are hundreds of such transgenes, some known to the public and some whose identities have been withheld as confidential business information. Field tests of these genetically engineered crops have been conducted for more than a decade in geographic regions where seed production occurs, and the transgenes in these tests represent a potentially large source of contamination.

This study's results should intensify the concerns of consumers who want to avoid genetically engineered foods for ethical, religious, or other reasons. In the United States, where purveyors of food need not disclose the presence of genetically engineered components, such consumers are already deeply frustrated by the lack of information in the marketplace. Contamination of traditional crop varieties with genetically engineered seeds and transgenic sequences only increases the difficulties consumers face.

In the trade arena, the study underscores the point that as long as the United States grows substantial acreages of engineered crop varieties and does not alter its commodity system (including the seed production system), it will not be able to provide uncontaminated commodity grain or oilseed products for any purpose. The lack of this capacity limits the attractiveness of U.S. products in the international marketplace.

Future concerns: pharm/industrial crops

The most urgent concern arising from this study does not relate to the current generation of products but to future products, in particular pharm and industrial crops. Many of these gene products would obviously be harmful if they were to appear at high levels in food or the environment. If, as the study suggests, the current seed production process is porous to contaminants, it offers a wide conduit through which the genes for pharm and industrial products may find their way into our food and feed systems or environment.

Industry and policy makers interested in pharm and industrial crops should receive this pilot study's message as a wake-up call: The seed supply for major food crops in the United States is vulnerable to contamination with drugs and industrial substances.

The result of such dangerous substances moving from seeds to consumers could be a disaster for human health. In addition, the economic impact of such an incident would ripple through the U.S. food chain, affecting millers, crushers, and retailers. The possibility that exported grain could be contaminated with substances such as drugs or plastics would further unnerve already wary foreign customers. In short, a contamination crisis similar to StarLink but involving drugs or industrial chemicals could set back, and perhaps even permanently derail, the U.S. agricultural biotechnology industry.

Industry and policy makers interested in pharm and industrial crops should receive this pilot study's message as a wake-up call: The seed supply for major food crops in the United States is vulnerable to contamination with drugs and industrial substances. Until we begin to address the problem of seed contamination, we must assume that pharm and industrial genes introduced into crops could become low-level contaminants of non-engineered seeds (or even other

genetically engineered seeds). As time passes and more transgenes for drugs and industrial chemicals are engineered into plants and tested in the environment, seeds may accumulate higher levels and a greater variety of these foreign genes and sequences.

Time to act

The current government approach to the contamination of bulk grain and oilseed products, landraces, or seeds appears ostrich-like: putting heads in the sand and hoping the phenomenon will go away.

But it will not.

Potential buyers of U.S. export products care about engineered contaminants for a number of legal, cultural, and other reasons and have plenty of other sellers to whom they can turn if the United States cannot meet their demands. From a health and environment standpoint, concerns cannot be written off simply because the levels of transgenically derived sequences are low; novel bioactive substances synthesized in transgenic pharm crops can do damage even at low levels. Moreover, transgenes that escape from the agricultural setting can be propagated in the environment and, in some cases, their levels could increase as a result of natural selection.

The fact—and possible consequences—of contamination can no longer be ignored. These concerns, especially where untested, unapproved substances intended as drugs or industrial chemicals are involved, hang like an ominous cloud over the future of agricultural biotechnology and the global food system.

RECOMMENDATIONS

The contamination of seeds of traditional crop varieties with transgenically derived DNA sequences must be addressed right away. The Union of Concerned Scientists recommends the following actions:

1. **The USDA should sponsor a full-scale investigation of the extent, causes, and impacts of contamination of the traditional seed supply by transgenically derived DNA sequences.**

The USDA should follow up this pilot study with a full-scale investigation of the extent, causes, and impacts of contamination of the traditional seed supply by DNA sequences originating in genetically modified organisms. This government-sponsored investigation should include traditional varieties of cotton, corn, canola, soybeans, and wheat, as well as fruits and vegetables for which genetically engineered varieties have been field tested. Although it has not been commercialized, genetically engineered wheat has been extensively field tested without stringent measures in place to guard against seed contamination. The USDA should look for contaminants originating in

The fact—and possible consequences—of contamination can no longer be ignored. These concerns, especially where untested, unapproved substances intended as drugs or industrial chemicals are involved, hang like an ominous cloud over the future of agricultural biotechnology and the global food system.

transgenic crop varieties that are being field tested, as well as in those that have been commercialized.

The investigation should encompass a sufficient number of samples taken from many seed sources in many parts of the country to ensure that its results will be representative of the general state of the traditional seed supply. The sample sizes should be large enough to provide reliable estimates of the extent and levels of contamination.

To address and eventually control contamination of the traditional seed supply by transgenically derived sequences, it is important to know why and where contamination occurs. There are basically two potential sources of contamination: physical mixing and outcrossing, both of which can occur at a number of points within the seed production process. New research should assess how mixing and outcrossing contribute to seed contamination across the entire spectrum of activities associated with seed production.

Special attention should be paid to understanding the points at which seed production would be vulnerable to contamination by pharm and industrial crops. That will allow scientists to devise strategies to control and prevent contamination in the future (see Recommendation 2).

The needed research is extensive. It must encompass seed production of major commodity crops at corporations, universities, on farms, and among national and international institutions. We recommend that the USDA fund the National Academy of Sciences Standing Committee on Agricultural Biotechnology, Health, and the Environment for the purpose of convening an expert panel to develop the scope and agenda for this research.

2. The USDA, FDA, EPA, and appropriate coordinating elements of the federal government should amend the regulations for transgenic pharm and industrial crops to ensure that the seed supply for food and feed crops is not contaminated at any level with drugs, vaccines, plastics, or related substances.

Protection of U.S. food and feed crops, as well as bulk food products, should be given the highest priority by the federal government in the coming year. We recommend that the USDA, FDA, EPA, and appropriate coordinating elements of the government amend pharm and industrial crop regulations to ensure that the seed supply for food and feed crops is completely protected against contamination with non-food transgenes and transgene products such as drugs, vaccines, plastics, and related substances.

This is a rigorous standard that is best achieved if pharm and industrial crops are regarded as a drug-manufacturing activity rather than a sideline of commodity crop production. Complete protection of the food supply against pharm and industrial crop contamination may not be achievable if food crops continue to be used as pharm and industrial crops.

Pharm and industrial crops have been planted without adequate control for more than a decade now, and only recently has the federal government awakened to the need for stronger regulation. The USDA recently imposed more rigorous containment procedures on the growing of pharmaceutical and industrial crops, but these new regulations do not even mention, much less address, the issue of seed supply contamination.

The USDA must amend existing pharm and industrial crop rules to deal with this issue and establish new restrictions based on an understanding of the points at which the seed production system is vulnerable to contamination. Such understanding will be the fruit of the additional studies recommended above. In the meantime, we recommend that the USDA immediately require short-term protections for the seed supply, such as

requirements that pharm and industrial crops not be grown on or near farm operations that also produce seed.

3. The USDA should establish a reservoir of seeds for non-engineered varieties of major food and feed crops free of transgenically derived sequences.

If the seed supply for major crops continues to be contaminated with genetic sequences derived from transgenic crops, it will become increasingly difficult to remove them from food, feed, or industrial systems should that become necessary or desirable. We believe the minimally prudent course is to have the USDA establish a reservoir of seeds for non-engineered varieties of major food and feed crops free of engineered sequences.

The appeal of a seed reservoir is that we would not be committing ourselves to a single path before we are sure it is the right one. If something does go wrong with genetic engineering, we will be able to shift onto a new course. Moreover, as discussed earlier, there are now and will likely continue to be trade and marketing advantages in our ability to reliably produce non-engineered products, but this will require the availability of uncontaminated seed for traditional crop varieties.

Setting up a reservoir of traditional seeds virtually free of engineered contaminants a decade and a half after the introduction of transgenic crops will be a challenge, but it is achievable. With careful attention to seed sources and strict new protocols for seed production, it should be possible to create breeder seed supplies that are free of genetically engineered sequences. Even if restoring the seed supply to a completely pristine state proves impossible, it will still be important to set up a seed reservoir with the lowest achievable amounts of contamination. The quantity of contamination matters; low levels of one or two transgenes are far better than high levels of hundreds of transgenes, especially pharm and industrial genes.

We recommend that the USDA develop a program that would ensure an uncontaminated supply of seeds for a long enough period to give us confidence in this new technology. Although any number is arbitrary, we suggest that 30 years might be appropriate.

4. The USDA and land-grant (agricultural) universities should reinvigorate the public plant breeding establishment to help ensure a supply of pure seed of traditional crop varieties.

One of the major trends of the last century has been the transformation of plant breeding from a publicly supported activity to a private one. Since private breeding is now conducted primarily by a handful of transnational companies, and those companies have switched almost completely to genetically engineered varieties of crops, a reinvigorated public plant breeding establishment is vital to the continued development of non-genetically modified varieties for commodity crops.

For public plant breeding to flourish, the USDA and land-grant universities must acknowledge the importance of plant variety development outside the confines of private corporations. They need to support genetic engineers who want to investigate crops and pursue projects that do not receive industry support. Even more urgently, the USDA needs once again to train classical breeders —as well as the soil scientists, plant pathologists, and agronomists on whom they depend—to provide the expertise necessary for the continued provision of non-genetically engineered seed. Public plant breeders would assist and cooperate with the Consultative Group on International Agricultural Research, international plant breeding institutions, farmer groups, and the many

volunteer "seed savers" who also participate in the global seed-producing enterprise.

5. The Association of Official Seed Certifying Agencies (AOSCA) should establish a national standard for breeder and foundation seed of traditional crop varieties: no detectable level of contamination by transgenes and associated sequences originating in genetically engineered crops.

Breeder and foundation seeds for commercial crops are key elements of our food and feed system. To give farmers opportunities to meet the demands of diverse domestic and global marketplaces, and to help create a national reservoir of non-engineered seed, it is important for the seed industry to establish standards assuring seed purchasers that non-transgenic seeds free of modified sequences are available.

We therefore recommend that the AOSCA establish a national standard of no detectable transgenically derived sequences in the breeder and foundation seeds for non-engineered varieties of major crops. The standard should specify appropriate tests such as PCR or other state-of-the-art methodologies.

6. The USDA, the organic agriculture community, land-grant universities, and plant breeders should develop new policies and programs to provide organic agriculture with pure seeds of traditional crop varieties.

Organic farming is one of the fastest-growing sectors of American agriculture, leading the way in the development of value-added food systems and providing new and growing opportunities for all sizes of farm operations. If organic agriculture is to reach its maximum potential, the USDA, land-grant universities, plant breeders, and the organic agriculture community itself should develop policies and programs that will ensure food and feed meet federal and international organic standards and any additional demands imposed by buyers of organic grain.

Essential to that effort is a guaranteed supply of uncontaminated seed for traditional crop varieties. The best way to provide this seed is in the context of partnerships among growers, public plant breeders, and agricultural scientists put together to select, test, and propagate seed tailored to the needs of organic agriculture. Promising initiatives along these lines are under way at Cornell and a handful of other universities mentioned in Chapter 3. We recommend adding the provision of seed for organic producers to the mission of these enterprises, and giving them the resources to accomplish this task.

Of course, while necessary, the provision of uncontaminated seed for organic agriculture is not sufficient to guarantee organic food and feed free of genetically engineered contaminants. That requires additional measures to address the problem of pollen inflow from engineered crops on neighboring fields. Individual organic farmers cannot stop this unwelcome arrival of pollen, which can degrade the quality of their products and put their certification as organic growers in jeopardy.

In the meantime, we recommend that consumers continue to purchase organic foods and support organic agriculture. Despite their best efforts, some organic producers may occasionally end up with products containing low levels of genetically engineered sequences, but this is the exception, not the rule. Organic producers are working hard to control sources of contamination and certified organic food remains the best marketplace option by far for consumers who demand uncontaminated products.

7. The USDA, the organic and biotechnology industries, and national growers' associations,

among others, should sponsor a series of meetings to begin addressing how those sectors of U.S. agriculture that have adopted transgenic crops and those threatened by contamination with transgenically derived DNA sequences from those crops can coexist.

Widespread use of transgenic crops will inevitably result in the transfer via pollen of engineered sequences and traits to compatible crops in nearby fields. If the growers of those nearby crops are attempting to harvest a product free of genetically engineered sequences, this unwanted contamination can have serious economic consequences. Whether facing an exacting customer in South Korea or an organic certifier, farmers in the receiving fields risk losing money if they try to market their contaminated crop.

This situation creates tension among producers. Who will accept responsibility and/or legal liability for the economic losses? Who will be accountable for the predictable results of choosing particular varieties? What sort of testing is currently done by growers and is there a way of spreading the costs of that testing?

European countries have identified the coexistence of agriculture sectors affected by the use of transgenic crops (both positively and negatively) as an important step to a prosperous and safe future, and set up a series of workshops to address these problems. Coexistence issues extend beyond seed production, but a series of similar conferences encompassing seed production would also have great value in the United States, particularly if they were sponsored by stakeholder groups including the organic community, national growers' associations, land-grant universities, and the USDA.

8. Private seed companies in the United States should periodically test their seed stocks, especially breeder and foundation seed and parental inbred lines, for the presence of transgenically derived DNA sequences. They should then make public the extent to which the seeds of the traditional varieties they market are free of transgenically derived contaminants.

Private seed companies in the United States could play a leading role in the effort to cleanse the seed supply for traditional varieties of crops by periodically testing their own breeder and foundation seed and parental inbred lines for the presence of transgenic seeds and transgenically derived sequences. In conjunction with that effort, these companies should then publicize their results.

The aggregate of the published results would provide a rough indication of the extent to which the U.S. supply of seeds for traditional varieties is contaminated and the progress being made in reducing contamination. Companies whose foundation and breeder seed stocks and parental inbred lines are free of transgenically derived DNA sequences should be proud to make that fact public.

Appendix A

Plant Breeding and Seed Production in Corn, Soybeans, and Canola

Below is a brief discussion of plant breeding and seed production in corn, soybeans, and canola.[95] See Figure 1-1 (p. 8) for a simplified diagram of the steps involved in the breeding and commercial seed production of a new crop variety.

DEVELOPING NEW COMMERCIAL VARIETIES

Until roughly the last 100 years, most plant breeding in the United States was undertaken by farmers, and in much of the world, farmers remain the plant breeders for important crops. Even in the United States, where commercial breeding is well established, plant breeding by farmers and gardeners continues to flourish.[96]

Early on in agriculture, farmers selected plants with favorable characteristics and saved their seed to plant in subsequent growing seasons. These farmer-selected plant types are called landraces.

Modern plant breeders, capitalizing on dramatic advances in genetics in the twentieth century, have raised plant breeding to a new level of sophistication. With the ability to identify, categorize, and characterize the genetic material of plants, breeders can select plants that have valuable new characteristics, cross-breed them with other varieties that have important agronomic traits, and find among the offspring plants exhibiting new combinations of desirable traits—in some cases, traits better than either parent. Promising offspring are tested and those that perform well in the field are sent into commercial seed-production processes. While still an art in some ways, traditional plant breeding has proved to be immensely successful and is responsible, to a great extent, for the significant productivity gains achieved in agriculture in the last century.

Sources of new traits

Farmers and commercial breeders rely primarily on the natural recombination resulting from sexual reproduction as the source of new traits for their breeding work. Sexual reproduction in plants involves the production of offspring through the combination of pollen from the male parent and eggs from the female parent. This process mixes genetic sequences from different parents, and every generation produces new combinations, some of which result in valuable traits such as increased yield or synchronous growth. Plants expressing these new traits are the raw material for a breeding program.

During the last two decades, genetic engineering techniques have begun to provide plant breeders with another source of new traits: genes taken from unrelated organisms. Methods such as mutagenesis, which induce changes in plant genes using chemicals or radiation, have been tried in the past but are rarely used anymore. Promising new approaches involving combinations of breeding and sophisticated genomic analysis,

95 We are grateful to Dr. Kendall Lamkey, professor, Department of Agronomy, Iowa State University, for helpful information on breeding and seed production, particularly in corn. For additional information, see Wych, R.D. 1988. Production of Hybrid Seed Corn. In *Corn and Corn Improvement*, agronomy monograph 18, ed., G.F. Sprague and J.W. Dudley, 565-607. Madison, WI: American Society of Agronomy; and Fehr, W.R. 1987. Breeding Methods for Cultivar Development. In *Soybeans: Improvement, Production, and Uses*, agronomy monograph 16, ed., J.R. Wilcox, 249-293. Madison, WI: American Society of Agronomy.

96 See Seed Savers Exchange at *http://www.seedsavers.org/wholepgs/Mainpgs/aboutus.htm* and Seed Savers Network at *http://www.seedsavers.net*.

though in the early stages of development, may become important in the future.

Testing new commercial varieties

Though some early steps in the breeding process of crops such as wheat and oats can be done in greenhouses, most breeding of traditional varieties of crops including corn and soybeans—which are not amenable to breeding in greenhouses—is done in the field. When transgenic varieties are being developed, the genetic engineering phase must of course be done in the laboratory, but once the genetic engineers have a plant expressing a transgenic construct, traditional breeders take over and complete the variety development process.

Breeders evaluate the new plant material in field tests, which may run from one to hundreds of acres and may be conducted in several different geographic locations to determine whether the varieties perform well under a range of environmental conditions. The varieties that perform the best in these field tests go into the commercial seed-production process.

PRODUCING SEED FOR NEW COMMERCIAL VARIETIES

In general, the seed industry produces seeds for two kinds of varieties: pure-line and hybrid. Pure-line varieties closely resemble their parent lines, and can be harvested and planted year after year with the expectation that plants with desirable characteristics typical of the parent variety will re-emerge each year. By contrast, hybrid offspring are strikingly different from their parents, and the seeds they produce cannot be saved and planted without losing desirable traits.

Virtually all commercial corn seed in the United States is hybrid—the product of controlled pollination. Soybean and canola seeds are sold in both pure-line and hybrid varieties, with most being pure-line. Although the major stages in seed production are the same for both, there are important differences discussed below.

Seed production may occur in the United States or abroad. Companies often want to take advantage of seasonal differences above and below the equator to produce seeds between growing seasons in North America. Nevertheless, much of the seed production takes place in the same region as commercial production of the crop. Nestled among the fields growing commodity corn and soybeans in Iowa and Illinois, for example, are fields devoted to corn and soybean seed production. Substantial canola seed production occurs in North Dakota, the site of most commercial U.S. canola production.

Pure-line seed production: soybeans and canola

Producing seed for non-hybrid varieties is a straightforward multiplication process beginning with small amounts of highly pure breeder seed and culminating two or three generations later with large quantities of seed to sell to farmers. For economic reasons, each generation of seed is grown under containment conditions less stringent than the preceding generation, resulting in a final commercial class of seed that is less pure than the original breeder seed.

Each step is given a class name that indicates to seed specialists and farmers the stringency under which the seed was produced and, hence, the purity of the seed. As noted in the following section on seed purity, certifying agencies set specific, numerical purity standards (and the procedures needed to achieve those standards) for each class in various crops. (See Table A-1 for examples of corn, soybean, and canola seed standards.)

Seed production for a new variety begins with breeder seed, which is produced and controlled by the plant breeding institution that developed the

new variety. Breeders take great care during seed production to prevent contamination.

The next step is to produce foundation seed. A small amount of breeder seed is planted and grown under less stringent controls to generate a larger amount of foundation seed. This seed may be used to produce additional foundation seed or the next class of seed: registered. Though some companies sell registered seed to farmers, more often they go one step further and produce larger amounts of certified seed. Companies may contract with farmers to grow foundation, registered, and certified seed.

The final stage in the production of commercial seed for farmers involves the following steps: sowing the seed, maintaining the crop during the growing season, harvesting the seed, then transporting, drying, cleaning, bagging, and storing the harvested seed until it will be shipped to seed retailers.

Hybrid seed production: corn

Hybrid seed production requires a more complicated approach in order to produce seeds exhibiting what is known as "hybrid vigor." This term refers to the superior traits exhibited by the offspring (hybrids) of two parents that lack those traits. This phenomenon is quite common in corn, which is one reason why hybrid seed is now the norm in corn seed production. Hybrid vigor is lost if seeds harvested from the hybrids are saved and planted the next year.

To generate commercial seeds with hybrid vigor, corn seed producers must plant large acreages of the two different parental lines, called inbreds. Producing enough seeds for the inbred lines begins with breeders. Once they have developed the new inbred lines for the new hybrid corn variety, they generate breeder seed for these lines under strict confinement measures.

Using breeder seed, the next step is to increase the amount of inbred seed. The new generation of inbred corn seed is termed foundation seed and, like the foundation seed of pure-line varieties, is typically produced under conditions less stringent than those for breeder seed. Foundation seed is then used in subsequent growing seasons to increase the amount of foundation inbred seed. Some companies refer to this process as parent seed production because foundation inbred seeds are the parents of hybrid seeds.

Once a company has enough foundation inbred seed, it begins producing hybrid seed in commercial quantities. Companies must ensure that all the seed produced during this stage results from the combination of two selected parents. To

Table A-1 **Association of Official Seed Certifying Agencies (AOSCA) Standards for Classes of Corn, Soybean, and Canola Seed***

	Hybrid Corn	Soybeans			Canola		
	Certified**	Foundation	Registered	Certified	Foundation	Registered	Certified
Pure seed (minimum)	98.0%	No standards	98.0%	98.0%	99.0%	99.0%	99.0%
Contaminant:							
Inert matter (maximum)	2.0%	No standards	2.0%	2.0%	1.0%	1.0%	1.0%
Weed seed (maximum)	0.0%	0.05%***	0.05%***	0.05%***	7 per lb.***	16 per lb.***	25 per lb.***
Total other crop seed (maximum)	No standards	0.2%	0.3%	0.6%	0.05%	0.1%	0.25%
Other varieties (maximum)	0.5%	0.1%	0.2%	0.5%	0.05%	0.1%	0.25%

*Adapted from AOSCA. 2001. Genetic and Crop Standards, pp. 2-29, 2-36, and 2-98. On the AOSCA website at *ftp://www.aosca.org/geneticstandards.pdf*, accessed on September 24, 2003.
**AOSCA recognizes only one class (certified) for hybrid corn seed.
***Includes zero tolerance for certain weeds.

begin, the parental inbreds are planted near one another, but the female parent must be prevented from pollinating itself by eliminating its ability to produce pollen. This is accomplished by mechanically removing its pollen-producing organs (tassels) or rendering it genetically sterile. The female inbred parent may then be wind-pollinated by the nearby male inbred parent or hand-pollinated.

The rest of the hybrid seed production process is very similar to that for pure-line varieties: at the end of the growing season, the hybrid seed is harvested, transported, dried, shelled, cleaned, bagged, and stored.

Contamination during seed production

Whether the result is hybrid or non-hybrid seeds, the process of variety development and seed production offers numerous opportunities for commingling of seeds and traits. This can occur through both physical mixing and cross-pollination.

Physical mixing opportunities arise during the planting of parent lines and the harvesting, sorting, handling, storage, or cleaning phases of seed production. Cross-pollination between plants can occur during the propagation of the parental lines and at several steps in the production of hybrid or pure-line seed. When stray pollen finds its way to receptive plants, the seeds produced may carry unwanted genetic sequences.

SEED PURITY STANDARDS

In the United States, the Association of Official Seed Certifying Agencies (AOSCA) establishes standards for seed purity that vary according to the kind of contaminant involved, the crop in which the contaminant is found, and the level of purity needed. For example, zero-tolerance standards apply to weed seeds in certified hybrid corn seed, while low levels of contaminating seeds of other crops (0.2 to 0.6 percent) are allowed in soybean seeds, depending on the class of seed (Table A-1, p. 59).

AOSCA recognizes the four levels of purity, or seed certification classes, mentioned above (breeder, foundation, registered, and certified) and sets specific procedures under which each level can be achieved during the seed production process.[97] These procedures typically involve restrictions on crops previously grown in seed production fields, minimum distances between seed production fields and nearby crops, and inspections of fields and seeds. The levels of purity achieved for each class vary from crop to crop and are set specifically for each crop. Not all classes exist for all crops; for example, there is only one class of hybrid corn: certified.

Although genetically modified varieties of a crop that are allowed on the market can be considered seed contaminants to the same extent as any other variety, engineered sequences in traditional seed are not currently considered contaminants for which standards have been set.

97 Association of Official Seed Certifying Agencies (AOSCA). 2001. Genetic and Crop Standards. On the AOSCA website at *http://www.aosca.org/genetic standards.pdf*, accessed on September 24, 2003. The website offers more information on AOSCA and the procedures required for various classes of certified seed in a variety of crops.

Appendix B

Transgenes and Transgenic Traits Listed in USDA Records of Field Tests of Genetically Engineered Corn, Soybeans, and Canola

Commercialized varieties of genetically engineered crops are not the only sources of seed contamination. Prior to commercialization, transgenic varieties are tested for several years in open fields, a practice that offers many opportunities for seed mixing and outcrossing.

Tables B-1 through B-6 list many transgenes and transgenic traits that have been field tested in the United States and may have moved into the seed supply. The identities of many other transgenes and traits that have also been field tested and may have moved into the seed supply are not listed because companies are allowed to withhold that information from the public as confidential business information (CBI).

Since 1987, corporations and university researchers have conducted thousands of field trials of genetically engineered plants in the United States. The U.S. Department of Agriculture (USDA), which oversees the tests, makes information on the trials available to the public through a database maintained by the Information Systems for Biotechnology (ISB) at Virginia Polytechnic Institute and State University.[98] Currently, that database contains nearly 10,000 records of field tests of engineered plants.

Each record consists of a number of fields containing information about the trials, including the recipient crop, the transgenes engineered into the crop, the traits conferred by those transgenes, the institution sponsoring the tests, and the states where the tests have been or are to be conducted. The records are compiled from information submitted to the USDA by those companies or universities seeking to conduct trials. Depending on the nature of the crop-gene combination and the intended use of the engineered crop, these submissions are either notifications of intent or requests for permission to conduct field tests.

Of the nearly 10,000 records on transgenic crops, more than half (5,528) concern field tests of the three crops that are the subject of this report. As of December 15, 2003, the USDA had acknowledged notifications or permitted field tests for 4,312 corn submissions, 711 soybean submissions, and 185 canola submissions (listed as rapeseed in the database).

Tables B-1 through B-6 list the transgenes and transgenic traits documented in USDA records of all tests of transgenic corn, soybeans, and canola that have been acknowledged or permitted by the department since 1987.[99] The information in the tables (which do not include records of submissions that are pending or have been withdrawn, denied, or voided) is taken directly from USDA records available on the

98 Information Systems for Biotechnology (ISB). 2003. Field Test Releases in the U.S. Blacksburg, VA: Virginia Polytechnic Institute and State University. On the ISB website at *http://www.isb.vt.edu/cfdocs/fieldtests1.cfm*, accessed on December 15, 2003.

99 We are grateful to the ISB staff for conducting special searches on December 15, 2003, that provided the information for the tables in this appendix.

ISB website, and is complete as of the access date (December 15, 2003).

As mentioned above, in a substantial portion of the records, the submitter has withheld information—including the names of the transgenes being tested—as CBI. As a result, the tables are far from a complete listing of the transgenes that have been field tested and may have moved into the seed supply. The percentage of records withholding the names of one or more transgenes is indicated below the tables that list transgenes in corn (Table B-1), soybeans (Table B-2), and canola (Table B-3).

Table B-1 **Transgenes Listed in USDA Records of Field Tests of Genetically Engineered Corn**

3-ketothiolase	Delta-12 desaturase antisense	Nopaline synthase
ACC synthase	Dihydrodipicolinate synthase	NptII
Aceto acetyl-CoA reductase	Dihydrodipicolinate synthetase	Nucleosome assembly factor A silencing
Acetolactate synthase	DNA adenine methylase	Nucleosome assembly factor C silencing
Acetyl CoA carboxylase	DNA methyltransferase	Nucleosome assembly factor D silencing
Acetyl CoA carboxylase antisense	DNA methyltransferase silenced	O-methyltransferase
Adenine methylase	Drug resistance protein (MRP29) antisense	Opaque 2
ADP glucose pyrophosphorylase	Enterotoxin subunit B	P regulatory gene
Albumin	EPSPS	P transcriptional activator
Aldehyde dehydrogenase	Esterase	P1 regulatory gene
Alpha-hemoglobin	Fertility restorer gene (rf2a)	P1 transcription factor
Amino polyol amine oxidase	Fertility restorer gene 2a	Phosphinothricin acetyl transferase
Amylase	Flavin amine oxidase	Polycomb group protein gene silenced
Anthocyanin regulatory gene	Flavonol 3-hydroxylase	Polycomb protein enhancer gene silenced
Anti-mutator gene B	Fructosyl transferase	Polyhydroxybutyrate synthase
Antibody (common cold)	G glycoprotein	Procollagen
Antibody (tooth decay)	Global transcription factor A silenced	Prolamin binding factor
Antifungal protein	Global transcription factor C silenced	Protein kinase
Aprotinin	Global transcription factor E silenced	Proteinase inhibitor I
Aspartokinase	Glucanase	Proteinase inhibitor II
B cell lymphoma related gene X (Bcl-xl)	Glutamate dehydrogenase	Pyruvate decarboxylase
B-glucuronidase	Glutathionine transferase	R gene transcription factor
B-Peru anthocyanin regulatory gene	Glutenin	R regulatory gene
B-Peru transcription factor-silenced	Glycogenin	Recombinase
B1 regulatory gene	Glycogenin antisense	Red fluorescent protein
B1 transcription factor	Glyphosate oxidoreductase	Replicase
Barnase	gp120 (glycoprotein 120)	Retinoblastoma 1 tumor suppresor antisense
Barstar	Green fluorescent protein	Retinoblastoma-related protein-silenced
Beta-hemoglobin	Helper protein mudrB	Ribonuclease
Branching enzyme (TB1)	Helper protein mudrB antisense	Ribosome inactivating protein
Brazzein	Histone acetylase gene silenced	Saccharopine dehydrogenase
Bromodomain protein gene silenced	Histone acetyltransferase gene silenced	Seed storage protein
C1 regulatory gene	Histone deacetylase	Self incompatibility
C1 transcription factor	Histone deacetylase silenced	Serum albumin
C1 transcriptional activator	Histone H1 gene silenced	SET domain protein gene silenced
CBI*	Homeotic regulatory gene (glossy 15)	Starch branching enzyme II
Cecropin	Homoserine dehydrogenase	Starch branching enzyme II antisense
Chitinase	Hygromycin phosphotransferase	Starch debranching enzyme
Chromatin remodeling complex-silenced	Isoamylase-type starch debranching enzyme	Starch synthase
Chromodomain protein gene silenced	Knotted-1	Starch synthase antisense
Citrate lyase	Laccase	Storage protein
Coat protein	Lectin	Sucrose phosphate synthase
Cry	Levansucrase	Sucrose synthase
Cry1F	Luciferase	Surface antigen
Cry9C	Lysine ketoglutarate reductase	T-URF13 mitochondrial
CryIA	Male sterility protein	Transcription regulator silenced
CryIA(b)	Methyl binding domain protein gene silenced	Transcriptional activator
CryIA(c)	Microtubule-associated protein (MAP4)	Transposon Mu1
CryIH	Mu transposable element	Transposon MuDR
CryIIA	Mu-1 transposable element	Transposon MuDR antisense
CryIIIA	Mu-A transposable element	Transposon Tn5
Cyclin dependent kinase	Mu-B transposable element	UDP glucose dehydrogenase
Cyclin dependent kinase inhibitor-silenced	MyB-IF35 transcription factor	Wheat germ agglutinin
Cystathionine synthase	N-terminal acetyl transferase silenced	Xylanase antisense
Cysteine proteinase inhibitors	Negative C transcription activator	Zein storage protein
Dehydroascorbate reductase	Negative R transcription activator	

***Confidential business information: 72% of the records do not disclose the names of one or more transgenes**

Table B-2 **Transgenes Listed in USDA Records of Field Tests of Genetically Engineered Soybeans**

10 kDa protein	Delta-15 desaturase	Omega 6 desaturase antisense
Acetolactate synthase	Delta-15 desaturase antisense	Oxalate oxidase
ACP acyl ACP thioesterase	Dihydrodipicolinate synthase	Oxygenase
Acyl-ACP thioesterase	Dihydrodipicolinate synthetase	Palmitoyl thioesterase
Aspartokinase	EPSPS	Palmitoyl thioesterase antisense
Aspartokinase II-homoserine dehydrogenase	Fluorescent protein	Phosphinothricin acetyl transferase
B-glucuronidase	Galactanase	Phosphoglucomutase
Calmodulin	Galactinol synthase	Protease
Casein	Glycinin	Protein kinase
CBI*	Homoserine dehydrogenase	Rps1-k resistance gene
Chitinase	Hygromycin phosphotransferase	Saccharopine dehydrogenase
Coat protein	Inositol hexaphosphate phosphohydrolyase	Seed storage protein
Conglycinin	Isoflavone synthase	Stearoyl ACP desaturase
CrylA(c)	Luciferase	Storage protein
Cyanamide hydratase	Lysine ketoglutarate reductase	Thioesterase
Cystathionine beta-lyase	Lysine ketoglutarate trypsin inhibitor	Transposon Tn5
Cystathionine synthase	Lysophosphatidate acyltransferase	UDP glucose glucosyltransferase
Delta-6 desaturase	NptII	UDP-glucose 4'epimerase
Delta-9 desaturase	Omega 3 desaturase	Zein storage protein
Delta-12 desaturase antisense	Omega 3 desaturase antisense	
Delta-12 saturase	Omega 6 desaturase	

***Confidential business information: 49% of the records do not disclose the names of one or more transgenes**

Table B-3 **Transgenes Listed in USDA Records of Field Tests of Genetically Engineered Canola**

Acetolactate synthase	CrylA(c)	Green fluorescent protein
Acetyl CoA carboxylase	Delta-9 desaturase	Hygromycin phosphotransferase
ACP acyl ACP thioesterase	Delta-9 desaturase antisense	Ketoacyl-ACP synthase
ACP thioesterase	Delta-12 desaturase	Ketoacyl-ACP synthase antisense
Acyl ACP antisense	Delta-12 desaturase antisense	Lysophosphatidic acid acetyl transferase
Acyl ACP desaturase	Delta-12 saturase	Lysophosphatidyl choline acetyl transferase
Acyl ACP desaturase antisense	Delta-12 saturase antisense	Nitrilase
Acyl CoA reductase	Delta-15 desaturase	NptII
Alanine aminotransferase	Delta-15 desaturase antisense	O-acyl transferase
B-glucuronidase	Desaturase 15 antisense	Oleayl-ACP thioesterase
B-ketoacyl-CoA synthase	Diacylglycerol acetyl transferase	Phosphinothricin acetyl transferase
B-ketoacyl-Coenzyme A synthase antisense	Dihydrodipicolinate synthase	Proteinase inhibitor I
Barnase	Elongase	Proteinase inhibitor II
Barstar	EPSPS	Reductase
CBI*	Fatty acid elongase	Sucrose phosphate synthase
Chitinase	Glucanase	Thioesterase
Coat protein	Glycerol-3-phosphate acetyl transferase	Thiolase
Cold regulated gene binding factor (CBF)	Glyphosate oxidoreductase	Trypsin inhibitor
CrylA(b)		

***Confidential business information: 47% of the records do not disclose the names of one or more transgenes**

Table B-4 **Transgenic Traits Listed in USDA Records of Field Tests of Genetically Engineered Corn**

Altered amino acid composition	Fusarium ear rot resistant	Oil profile altered
Altered maturing	Fusarium ear rot susceptible	Oil quality altered
Altered morphology	Fusarium resistant	Pharmaceutical proteins produced
Altered plant development	Gene expression altered	Phosphinothricin tolerant
Alternaria resistant	Germination increased	Photosynthesis enchanced
Animal feed quality improved	Glucuronidase expressing	Phytate reduced
Anthocyanin produced in seed	Glyphosate tolerant	Pigment composition altered
Anthracnose resistant	Grain processing improved	Pigment metabolism altered
Anthracnose susceptible	Gray leaf spot resistant	Polymer produced
Antibiotic produced	Gray leaf spot susceptible	Processing characteristics altered
Antibody produced	Growth rate altered	Protein altered
Aspergillus resistant	Growth rate increased	Protein levels increased
Botrytis resistant	Helminthosporium resistant	Protein lysine level increased
Capable of growth on defined synthetic media	Herbicide tolerance	Protein quality altered
Carbohydrate level increased	Imidazole tolerant	Protoporphyrinogen oxidase inhibitor tolerant
Carbohydrate metabolism altered	Imidazolinone tolerant	Recombinase produced
Carotenoid metabolism altered	Increased phosphorus	Rhizoctonia resistant
CBI	Increased stalk strength	Salt tolerance increased
Cell wall altered	Increased transformation frequency	Seed color altered
Cercospora resistant	Inducible DNA modification	Seed composition altered
Chloroacetanilide tolerant	Industrial enzyme produced	Seed methionine storage increased
Cold intolerant	Isoxaflutole resistant	Seed quality altered
Cold tolerant	Isoxazole tolerant	Seed size increased
Coleopteran resistant	Kanamycin resistant	Seed weight increased
Color altered	Leaf blight resistant	Selectable marker
Color pigment restored	Leaf spot resistant	Senescence altered
Color sectors in seeds	Lepidopteran resistant	Septoria resistant
Colorado potato beetle resistant	Lignin levels decreased	Smut resistant
Colored sectors in leaves	Lipase expressed in seeds	Southern rust susceptible
Common rust susceptible	Lysine level alterered	Southern corn leaf blight resistant
Corn earworm resistant	Lysine level increased	Southern corn leaf blight susceptible
Cre recombinase produced	Male sterile	Southwestern corn borer resistant
Cyanamide tolerance	Male sterile nuclear	Starch level increased
Cyanamide tolerant	Male sterile reversible	Starch metabolism altered
Dalapon tolerant	Maturity altered	Starch reduced
Development altered	MCDV resistant	Stewart's wilt susceptible
DNA synthesis altered	MCMV resistant	Storage protein
Drought tolerant	MDMV resistant	Storage protein altered
Ear mold resistant	MDMV-B resistant	Stress tolerant
Endosperm DNA synthesis altered	Metabolism altered	Sugar cane borer resistant
Environmental stress reduced	Methionine level increased	Sulfonylurea tolerant
Epidermal cells increased on juvenile leaves	Modified growth characteristics	Transposon elements inserted
European corn borer resistant	Mutator transposon suppressed	Transposon inserted
Expression optimization	Mycotoxin degradation	Transposon movement supressed
Eyespot resistant	Mycotoxin production inhibited	Tryptophan level increased
Fall armyworm resistant	Nitrogen metabolism altered	Visual marker
Fertility altered	Northern corn leaf blight resistant	Visual marker inactive
Flowering time altered	Northern corn leaf blight susceptible	Vivipary increased
Fumonisin degradation	Novel protein produced	Western corn rootworm resistant
Fungal post-harvest resistant	Nutritional quality altered	Yield increased

Table B-5 **Transgenic Traits Listed in USDA Records of Field Tests of Genetically Engineered Soybeans**

2,4-D tolerant	Fusarium resistant	Phytate reduced
Altered amino acid composition	Glyphosate tolerant	Phytophthora resistant
Altered maturing	Grain processing improved	Pollen visual marker
Altered plant development	Growth rate altered	Polymer produced
Animal feed quality improved	Imidazole tolerant	Protein altered
Antibody produced	Imidazolinone tolerant	Protein quality altered
Antiprotease producing	Increased protein levels	Recombinase produced
BPMV resistant	Increased transformation frequency	Salt tolerance increased
Bromoxynil tolerant	Industrial enzyme produced	SbMV resistant
Carbohydrate metabolism altered	Isoxaflutole resistant	Sclerotinia resistant
CBI	Isoxazole tolerant	Secondary metabolite increased
Cold tolerant	Kanamycin resistant	Seed composition altered
Coleopteran resistant	Lepidopteran resistant	Seed methionine storage increased
Cyanamide tolerant	Lysine level increased	SMV resistant
Development altered	Male sterile nuclear	Stanol increased
Dicamba tolerant	Methionine level increased	Sterols increased
Drought tolerant	Nitrogen metabolism altered	Storage protein altered
Ear mold resistant	Novel protein produced	Transformation frequency increased
Fatty acid level altered	Nutritional quality improved	Visual marker
Fatty acid metabolism altered	Oil profile altered	White mold resistant
Feed properties altered	Oil quality altered	Yield increased
Fumonisin degradation	Oleic acid content altered in seed	
Fungal susceptibility	Phosphinothricin tolerant	

Table B-6 **Transgenic Traits Listed in USDA Records of Field Tests of Genetically Engineered Canola**

Altered amino acid composition	Fungal post-harvest resistant	Oil quality altered
Bromoxynil tolerant	Glyphosate tolerant	Pharmaceutical proteins produced
CBI	Industrial enzymes produced	Phoma resistant
Cold tolerant	Lepidopteran resistant	Phosphinothricin tolerant
Coleopteran resistant	Lysine level increased	Polymer produced
Cylindrosporium resistant	Male sterile	Sclerotinia resistant
Erucic acid altered	Male sterile reversible	Seed composition altered
Fatty acid metabolism altered	Nitrogen metabolism altered	Sulfonylurea tolerant
Fertility altered	Nutritional quality altered	Visual marker
Fertility restored	Oil profile altered	Yield increased

Glossary

Biotechnology
Term referring to practical uses of living organisms. "Old" biotechnologies typically include processes such as fermentation (to make foods such as yogurt, cheese, bread, and beer), animal and **plant breeding**, and food and fiber production from plants and animals. "New" biotechnologies include modern techniques such as genetic engineering and cloning. The term biotechnology is often used interchangeably with the terms **genetic engineering** and **genetic modification**.

Breeder seed
Seed held most closely by breeders of new plant **varieties**. Breeder seed is the class of **certified seed** with the highest standards for purity and is the source for production of **foundation seed**.

Bt crop
Insect-resistant crop **variety** engineered to produce an insect toxin originally found in the soil bacterium *Bacillus thuringiensis.* YieldGard, NaturGard, KnockOut, and StarLink are trade names of some Bt-corn varieties.

Bt toxin
Insecticidal toxin produced by *Bacillus thuringiensis* bacteria. The **gene** for Bt toxin has been engineered into a number of **biotechnology** crops.

Center of diversity
Locale where the relatives of crops have the greatest genetic diversity in the form of **traditional varieties** and/or wild relatives.

Certified seed
Generically, seed that has been subject to certification by a seed-certifying agency. Classes of certified seed, listed from most to least pure, are **breeder**, foundation, registered, and certified.

Specifically, that particular class of certified seed typically produced from **registered seed**, but which also may be produced from **foundation seed** or other certified seed. Certified seed is usually the class of seeds sold to farmers and is typically the least genetically pure of the four classes of certified seed.

Construct
Assemblage of **genetic sequences** spliced together into a unit easily moved around by genetic engineers. Constructs typically include one or more **genes** for new traits (such as herbicide resistance and insect resistance) as well as **regulatory sequences** such as **promoters** and **terminators**.

Crop gene pool
All the **genes** in all the **varieties** of a crop, plus the genes of **landraces** and wild relatives that interbreed with the crop.

Cross-pollination
see **outcrossing**

Detection limit
Lowest level at which target **DNA** can exist in a sample and be reliably detected by **polymerase chain reaction (PCR)** methods. In this report, the detection limit is typically expressed as a percentage: the ratio of the number of transgenically derived **genomes** to the number of crop genomes times 100 percent.

DNA
Deoxyribonucleic acid, the linear macromolecule that makes up the genetic material of most organisms. DNA usually exists as a double-stranded helix.

Engineered construct
see **construct**

Event
Line of plants resulting from the insertion of a transgenically derived **construct** into the **genome** of a plant. Each insertion results in a different event, even when containing the same **gene**. Most of the events discussed in this report represent different constructs.

Expression
see **gene expression**

Fertilization
Combining male sex cells carried within **pollen** grains with female sex cells (eggs) to produce plant embryos. Fertilization triggers the formation of seeds, which contain embryos.

Foundation seed
Class of **certified seed** produced from **breeder seed** or other foundation seed under conditions that maintain high standards of genetic identity and purity. Foundation seed is the source of certified seed, either directly or as the source of **registered seed** that is then used to produce certified seed.

Gene
Functional unit of hereditary material (**DNA**) usually carried on chromosomes and passed from parent to offspring. A gene codes for proteins (the molecules that are responsible, alone or in combination, for traits exhibited by plants such as seed color and shape, height, and insect resistance).

Gene expression
Production of proteins coded for by **genes**.

Gene flow
The successful movement of **genes** from one population of plants to another, usually via **pollination**.

Gene product
Protein resulting from **gene expression**.

Gene splicing
see **genetic engineering**

Genetic element
see **genetic sequence**

Genetic engineering
Molecular-level techniques capable of combining **genes** and **regulatory sequences** and transferring them into an organism. These techniques, which may be used to transfer genes between unrelated organisms or to remove and rearrange genes within a species, are also called **transgenic**, **gene splicing**, and **genetic modification** techniques.

Genetic modification
Strictly speaking, any mode of altering the genetic composition of organisms. The term, especially in Europe, has come to refer more narrowly to modern gene transfer techniques and is used interchangeably with **transgenic**, **gene splicing**, and **genetic engineering** techniques.

Genetic sequence
Segment of **DNA** that codes for proteins or regulates their function.

Genetically engineered organism
Organism (or progeny of an organism) whose **genetic sequences** have been modified using molecular-level techniques. Such organisms are also referred to as **genetically modified** or **transgenic**.

Genetically modified organism (GMO)
see **genetically engineered organism**

Genome
The full set of **genes** and associated **DNA** characteristic of an organism.

GMO testing
Use of sophisticated biochemical methods to analyze food, feed, and other agricultural products for **genetic sequences** originating from engineered **varieties** (i.e., **genetically modified organisms**).

Herbicide-resistant variety
Plant **variety** resistant to the otherwise toxic effects of herbicides.

Hybrid variety
Offspring of two parent plants that differ from one another in one or more **genes** and often exhibit **hybrid vigor.** Such varieties typically do not breed true.

Hybrid vigor
Phenomenon whereby the offspring exhibit traits more desirable than either of the parents.

Identity-preserved (IP) system
Carefully controlled production and distribution system that segregates high-value crops from the time of planting to delivery to the end user.

Inbred crop
Pure-breeding line of plants that has undergone controlled **pollination** for a number of generations.

Landrace
Improved plants selected and maintained by farmers and typically found where crops have been grown for many generations. Landraces are not the products of modern **plant breeding** or **genetic engineering**.

Limit of detection
see **detection limit**

Limit of quantification
see **quantification limit**

Novel gene
see **transgene**

Outcrossing
Sexual reproduction between two different individual plants.

Pharm crop
Crop engineered to produce pharmaceuticals.

Plant breeding
Scientific discipline for producing new crop **varieties** using sophisticated, field-based selection and mating techniques.

Pollen
Dust-like material, produced by the male parts of flowers, that contains male sex cells.

Pollination
Transfer of **pollen**, most frequently accomplished by wind or insects, from the male part of a plant flower to the female part. If the pollen is compatible with the female part of the flower to which it has been transferred, pollination is followed by **fertilization**.

Pollination is sometimes used as shorthand for both pollen transfer and fertilization.

Polymerase chain reaction (PCR)
Technique used to determine whether a sample of plant tissue contains a particular **DNA** sequence. PCR relies on **primer sets** that home in on a particular target DNA sequence and a special DNA-copying enzyme (DNA polymerase) that makes enough copies of the target sequence for identification and measurement. See also **qualitative PCR**, **quantitative PCR**, and **semi-quantitative PCR**.

Primer set
Short pieces of **DNA** added to **polymerase chain reaction (PCR)** mixtures to "find" the pieces of target DNA that will be copied. Primer sets are synthesized to match sequences at the beginning and end of the target DNA, thereby defining the exact segment to be subsequently duplicated by a DNA-copying enzyme.

Promoter
Regulatory sequence of **DNA** that controls the process by which **genes** are translated into proteins. In addition to initiating the process, such sequences can also determine the amount of protein produced. The 35S promoter derived from the cauliflower mosaic virus, for example, is the most widely used promoter in crop **genetic engineering**.

Pure-line variety
Plants that are genetically identical and typically breed true (i.e., the progeny of **self-pollinating** pure-line varieties are indistinguishable genetically and in appearance from the parent **varieties**).

Qualitative PCR
Polymerase chain reaction (PCR) methods that determine the presence or absence of a specific target **DNA** sequence at a particular level of detection.

Quantification limit (QL)
Lowest level at which the amount of a target **DNA** sequence in a sample can be reproducibly measured. In this report, the quantification limit is typically expressed as a percentage: the ratio of the number of transgenic **genomes** to the number of crop genomes times 100 percent.

Quantitative PCR
Polymerase chain reaction (PCR) methods that estimate the relative amount of a target **DNA** sequence in a mixture of DNA molecules (at a particular level of quantification).

Registered seed
Class of **certified seed** generally produced from **foundation seed** under conditions that maintain certain standards of identity and purity. These standards are lower than those for foundation seed but higher than those for certified seed. Registered seed is generally a source of certified seed.

Regulatory sequence
Segment of **DNA** that controls the process by which cells manufacture proteins. **Promoters** and **terminators** are the most common regulatory sequences used in **genetic engineering**.

Self-pollination
Transfer of **pollen** from the male part of a plant flower to the female part of a flower on the same plant. After **pollination**, male and female cells combine to form embryos (**fertilization**). Soybean is a predominantly self-pollinating crop, while corn and canola are predominantly **cross-pollinating**.

Semi-quantitative PCR
Polymerase chain reaction (PCR) methods designed to determine in one analysis the presence or absence of a target **DNA** sequence and an estimate of its relative amount in a mixture of DNA molecules.

Stacked gene
One of two or more **transgenes** expressed in a genetically engineered **variety**, such as a cotton plant engineered to produce both a **Bt** toxin and a protein that enables the plant to resist glyphosate herbicides.

Terminator
Regulatory sequence of **DNA** that stops the process by which a protein is produced from a **gene**. The NOS terminator from the bacterium *Agrobacterium tumefaciens*, for example, is the most widely used terminator sequence in plant **genetic engineering**.

Traditional breeding
see **plant breeding**

Traditional variety
Crop **variety** with no history of **genetic engineering**. Traditional varieties are produced through **plant breeding** techniques that rely on selecting and mating parent plants possessing promising traits and repeatedly selecting for superior performance among their offspring.

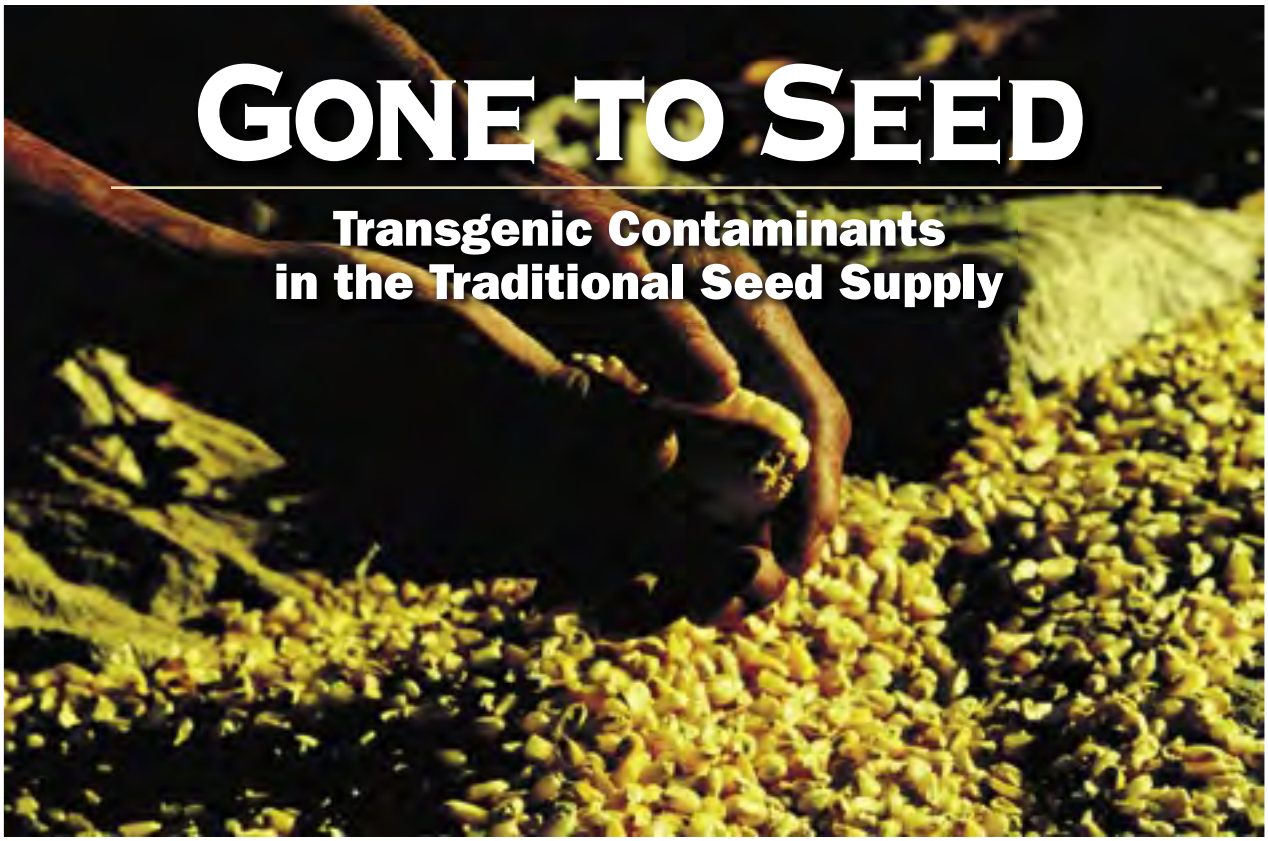
Transformation event
see **event**

Transgene
Gene transferred to an organism through **genetic engineering**.

Transgenic
see **genetic engineering**

Transgenically derived sequence
DNA sequence originating from a plant produced as a result of **genetic engineering**.

Variety
Subgroup of plants within a species whose genetic makeup and characteristics distinguish it from other varieties of the species. Crop varieties are often called cultivars, especially by agricultural scientists.



Gone to Seed

Transgenic Contaminants in the Traditional Seed Supply

Nothing is more fundamental to agriculture and our food supply than seeds. The variety, abundance, and safety of foods all depend on the availability and quality of seeds.

In *Gone to Seed*, the Union of Concerned Scientists (UCS) examines a new phenomenon that may threaten the quality of the traditional seed supply: contamination by DNA sequences used in genetic engineering. UCS conducted a small pilot study of seeds of traditional varieties of corn, soybeans, and canola purchased from the same retailers used by U.S. farmers. Laboratory testing showed the seeds are contaminated with low levels of DNA originating in genetically engineered varieties of those crops.

This report addresses the implications of seed contamination in several regulatory and policy contexts, including pharmaceutical-producing crops, trade, and organic food production. It then offers recommendations—to the federal government, seed companies, and agricultural universities, among others—for confronting this problem before it is too late.

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Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population

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Abstract. 1. The size of the Mexican overwintering population of monarch butterflies has decreased over the last decade. Approximately half of these butterflies come from the U.S. Midwest where larvae feed on common milkweed. There has been a large decline in milkweed in agricultural fields in the Midwest over the last decade. This loss is coincident with the increased use of glyphosate herbicide in conjunction with increased planting of genetically modified (GM) glyphosate-tolerant corn (maize) and soybeans (soya).

2. We investigate whether the decline in the size of the overwintering population can be attributed to a decline in monarch production owing to a loss of milkweeds in agricultural fields in the Midwest. We estimate Midwest annual monarch production using data on the number of monarch eggs per milkweed plant for milkweeds in different habitats, the density of milkweeds in different habitats, and the area occupied by those habitats on the landscape.

3. We estimate that there has been a 58% decline in milkweeds on the Midwest landscape and an 81% decline in monarch production in the Midwest from 1999 to 2010. Monarch production in the Midwest each year was positively correlated with the size of the subsequent overwintering population in Mexico. Taken together, these results strongly suggest that a loss of agricultural milkweeds is a major contributor to the decline in the monarch population.

4. The smaller monarch population size that has become the norm will make the species more vulnerable to other conservation threats.

Key words. Glyphosate, GMO, milkweed, monarch butterfly

Introduction

Monarch butterflies (*Danaus plexippus* L. Lepidoptera: Danainae) in the Eastern North American migratory population undergo a multi-generation annual cycle that includes wintering in central Mexico. In the spring, adults that have overwintered migrate north and reproduce in Texas and states to the north and east. Their offspring move farther north into much of the eastern half of the United States and southern Canada, and two to three more generations are produced (Cockrell *et al*., 1993; Malcolm *et al*., 1993; Prysby & Oberhauser, 2004). Most adults that emerge after mid-August are in a state of reproductive diapause (Herman, 1985; Goehring & Oberhauser, 2002) and migrate from the summer breeding range to their wintering grounds, where they remain until spring (Solensky, 2004).

Annual counts of the size of the overwintering population in Mexico indicate that the monarch population has been declining over the last decade and a half (Rendón-Salinas et al., 2011; Brower *et al*., 2011b). One possible explanation for this decline is that monarch production has been decreasing as a result of a reduction in the availability of the larval host plant. Monarch larvae feed primarily on milkweeds (genus *Asclepias*- Family *Apocynaceae*, subfamily *Asclepiodeae*). On the basis of milkweed cardenolide fingerprints, it has been estimated that 92% of the monarchs wintering in Mexico had fed as larvae on the common milkweed, *Asclepias syriaca* (Malcolm *et al*., 1993). Studies in Iowa found a large reduction in *A. syriaca* in corn (maize, *Zea mays*) and soybean (soya, *Glycine max*) fields from 1999 to 2009 (Hartzler & Buhler, 2000; Hartzler, 2010). It is likely that a similar reduction has occurred throughout the region where corn and soybeans are predominantly grown. Eighty per cent of both

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corn and soybeans are grown in the Midwest (USDA, National Agricultural Statistics Service, 2011c), which is composed of the states of North and South Dakota, Nebraska, Kansas, Missouri, Iowa, Minnesota, Wisconsin, Illinois, Indiana, Michigan, and Ohio. A study in 2000 (Oberhauser *et al*., 2001) found that monarchs heavily used milkweeds in corn and soybean fields. On the basis of stable isotope analysis, Wassenaar and Hobson (1998) estimated that half of the monarchs overwintering in Mexico in 1997 came from the Midwest. Thus, the Midwestern United States is at the epicentre of a reduction in milkweeds in agricultural fields and is also an area that has in recent history contributed a large component of the monarch population. In this study, we estimate the magnitude of this milkweed loss and its consequences for monarch production.

Milkweed in agricultural fields has long been a concern for farmers as its presence reduces yield (Bhowmik, 1994). In the 1970s and 1980s, milkweed infestation in agricultural fields was viewed to be on the increase with 10.5 million ha infested in the north-central states (Martin & Burnside, 1980). Herbicides have been increasingly used to control weeds in row crops. Many of these herbicides produce only moderate control of milkweed, but glyphosate, often referred to as Roundup™ (Monsanto, St. Louis, MO, USA), is more effective (Bhowmik, 1994; Pline *et al*., 2000). However, it also has a detrimental effect on crop plants, so until the development of genetically modified (GM) glyphosate-tolerant (Roundup Ready™, Monsanto) crop plants, herbicides other than glyphosate were used to control weeds. Glyphosate-tolerant soybeans were introduced in 1996 and had reached a 94% adoption level by 2011, and glyphosate-tolerant corn was introduced in 1998 and had reached a 72% adoption level by 2011 (USDA, Economic Research Service, 2011). Glyphosate use in soybeans went from 1.4 million kg in 1994 to 41.7 million kg in 2006 (the last year for which data are available and when adoption of glyphosate-tolerant soybeans was 89%) and glyphosate use in corn went from 1.8 million kg in 2000 to 28.5 million kg in 2010 when the adoption level was 70% (USDA, National Agricultural Statistics Service, 2011a,b).

The time period (1999–2009) over which the Iowa studies found a large reduction in *A. syriaca* in corn and soybean fields (Hartzler & Buhler, 2000; Hartzler, 2010) is coincident with the period when use of glyphosate herbicide increased in conjunction with the increased adoption of glyphosate-tolerant corn and soybeans. It is very probable that a similar milkweed reduction has occurred throughout the Midwest because adoption levels of herbicide-tolerant crops are similar throughout this region (USDA, Economic Research Service, 2011). How much milkweed loss does this represent on a landscape scale? To address this question, we need information on the density of milkweeds in different habitats and the landscape area covered by those habitats. Common milkweed tends to be found in habitats with a moderate degree of disturbance, including roadsides, pastures, old fields, prairies and agricultural fields (Bhowmik, 1994). Multiple data sets provide information on the density of milkweeds in different habitats over the last decade. The studies by Hartzler and Buhler (2000) and Hartzler (2010) surveyed a number of milkweed habitats in Iowa, including agricultural fields. Additionally, a number of Midwest volunteers in the Monarch Larva Monitoring Project (2011), hereafter referred to as MLMP, measured milkweed density in their non-agricultural observation patches over several consecutive years. Milkweed density data can be combined with published statewide land-use data to estimate the number of milkweeds in different habitats. Some of the data sets we use come from Iowa because for some parameters only Iowa data are available. However, we use data from the Midwest as a whole whenever possible and make the case that the resulting estimates of monarch production are representative of the Midwest.

What is the significance of the loss of milkweeds in agricultural fields for monarchs? To address this issue, we need to estimate annual monarch production in the Midwest over the last decade to determine whether there has been a significant downward trend. Obtaining data to estimate production is difficult, despite the fact that the monarch butterfly is such a well-studied species. One approach would be to use the number of migrants that come out of the Midwest at the end of the summer as a measure of production. A monarch tagging programme begun 20 years ago (Monarch Watch, 2011) has been tracking migrating butterflies. The number of monarchs tagged shows a decline from 2004 to 2010 (Brower *et al*., 2011a). However, it is difficult to obtain accurate measures of production from this tagging programme because of the variability among the years in the number of person-hours involved in capture and tagging, the fall conditions when tagging occured and the locations where tagging occured. Alternatively, one could use counts of the number of migrating monarchs passing particular locations where they tend to be funnelled because of passage over water or geography. Such counts have been made for over a decade in upper Michigan and New Jersey (Davis, 2011) but these sites do not monitor monarchs from the Midwest.

Rather than trying to count adults, another approach to estimating Midwest monarch production is to focus on the number of eggs and larvae found on milkweed plants. This requires monitoring many patches of milkweed in different habitats, including agricultural fields. Production can then be estimated from the average number of monarchs per plant in each habitat and the number of milkweeds in each habitat on the landscape. We have combined several existing data sets that provide this information. The MLMP (2011), which has been operational for over a decade, provides data on egg and larva density on milkweeds. MLMP volunteers are located throughout the monarch breeding range and monitor sites of their choosing weekly over the summer months, reporting the number of plants (stems) monitored and the number of eggs and larvae observed. They learn the procedures of the project through workshops, by reading directions on the project website (MLMP, 2011) and via communication with the project managers (Prysby & Oberhauser, 2004). The sites they monitor, however, are not in agricultural fields. But one of us (Pleasants) has monitored eggs and larvae on milkweeds in both agricultural fields and non-agricultural habitats for several years in central Iowa and a study with larger spatial scale quantified monarch density in both agricultural and non-agricultural habitats in 2000 (Oberhauser *et al*., 2001). We will make the case that the relative use of milkweeds in agricultural and non-agricultural habitats observed over those years can be extrapolated to provide

data on monarch use of agricultural milkweeds in years where only MLMP data exist. There is a question of what aspect of production to use to estimate monarch population changes. The latest stage for which we have density data, and thus which is closest to *actual* production of adult monarchs, is the fifth instar (L5, the last larval instar). However, there are many factors that can affect survivorship from egg to L5 that have nothing to do with milkweed availability, such as predation and weather. Our goal was to examine the effect of milkweed resource limitation on monarch production. Consequently, we chose to focus on eggs per plant that represents *potential* production.

Methods

Data sources for milkweed density

Habitats in which milkweeds are found include primarily roadsides, corn fields, soybean fields, pastures, old fields, and land set aside from farming and enrolled in the Conservation Reserve Program (CRP). CRP land is typically planted to a variety of cover plants including grasses and forbs. To estimate milkweed densities in these habitats, we used data from several sources: Iowa censuses carried out in 1999 and 2009 (Hartzler & Buhler, 2000; Hartzler, 2010), and data from some MLMP volunteers who measured milkweed density at their sites in several Midwest states. To calculate monarch production for each year, it is necessary to know how milkweed densities have changed over the last decade in non-agricultural and agricultural habitats.

Non-agricultural habitats. For roadsides, there was little observed change in milkweed density in Iowa between 1999 and 2009 (Hartzler, 2010) so we have assumed that milkweed density did not change in that habitat over the entire period of the analysis. Hartzler (2010) measured milkweed densities for CRP land and pastures in 1999 but not in 2009 so any change that may have occurred could not be determined from the Iowa data. However, a subset of MLMP volunteers (n = 16) measured milkweed density at their sites (which included natural areas, CRP land, pastures and old fields) for at least 4 years over this period (97 total observations). Measurements by individual MLMP volunteers did not cover the entire period but there were sufficiently long and overlapping sequences to provide a complete picture. Volunteers either measured the area of their site and did a complete count of milkweed stems, or used a modified belt transect to sample milkweed density in 100 1 × 1 m plots. We have used those data to estimate the change in milkweed density in CRP land and pasture land over the last decade.

For the data from the MLMP volunteers, we used log of milkweed density as the variate and used an SAS mixed model and restricted maximum likelihood estimation with fixed effects being 'habitat', 'year' and 'habitat by year'. We did not find a 'habitat by year' effect so we reran the analysis with this removed. There was a significant 'year' effect ($F_{1,85}$ = 9.35, P = 0.003). The slope of the regression (on a log scale) was −0.0536, which corresponds to a decline in density of 5.2% per year. We found no 'habitat' effect so we applied the same rate of decline to both CRP land and pastures (Fig. 1).

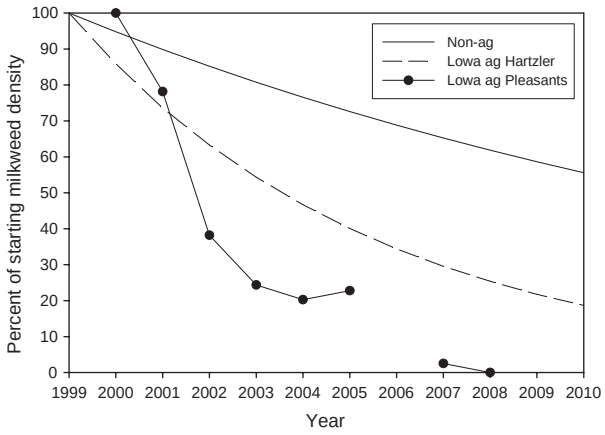

Fig. 1. Decline of milkweeds in agricultural and non-agricultural habitats. The line depicting the decline in non-agricultural habitats is based on a regression using data from MLMP volunteers. The line depicting the decline in agricultural habitats is based on an exponential decay function connecting the 1999 and 2009 values from the Iowa surveys (see Methods). Also shown is the proportional change in the number of milkweed stems in all monitored plots in seven agricultural fields in Iowa starting with 998 stems in 2000. The increase in milkweed stems observed in the agricultural sites in 2005 was attributed to the influence of fields where corn was planted 2 years in a row. Some agricultural fields received glyphosate herbicide treatment and others non-glyphosate treatment. No observations were made in 2006.

Agricultural habitats. We have values for milkweed density in Iowa agricultural fields for 1999 and 2009 (Hartzler & Buhler, 2000; Hartzler, 2010). To calculate milkweed density in fields for the intervening years, we have to make an assumption about the shape of the decline. Pleasants observed the change in the number of milkweeds in plots in seven agricultural fields in Iowa from 2000 to 2008 (Fig. 1). The observed decline is best described by an exponential decay function. Such a function is also consistent with more acres of glyphosate-tolerant corn and soybeans being planted each year over the last decade (USDA, Economic Research Service, 2011). We have therefore assumed that milkweed density in fields decreased as an exponential decay function from its 1999 value to its 2009 value (see Table 1). This corresponds to a 14.2% decline per year (Fig. 1). Other decline functions, ranging from a linear decline to a more precipitous exponential decay, had no significant effect on the overall results.

Data sources for land use

We obtained data on the acres occupied by roadsides and pastures on the Iowa landscape in 2002 from Lubowski *et al.* (2006) and, because no more recent data exist, we have assumed the

Table 1. Estimates of the amount of milkweed in non-agricultural habitats, agricultural fields and total milkweeds in Iowa from 1999 to 2010.

	Milkweeds in non-agricultural habitats				
Year	CRP hectares*	CRP mlkwds†	Pasture mlkwds‡	Roadside mlkwds§	Total non-ag mlkwds¶
1999	601	127.4	19.8	38.2	185.4
2000	647	130.1	18.8	38.2	187.1
2001	729	139.0	17.8	38.2	195.0
2002	755	136.4	16.9	38.2	191.5
2003	762	130.5	16.0	38.2	184.7
2004	767	124.5	15.2	38.2	177.9
2005	776	119.5	14.4	38.2	172.0
2006	793	115.7	13.6	38.2	167.5
2007	797	110.3	12.9	38.2	161.4
2008	733	96.2	12.3	38.2	146.6
2009	690	85.8	11.6	38.2	135.7
2010	663	78.2	11.0	38.2	127.4
Year	Milkweeds in agricultural fields				
	Total ag hectares**	Mlkwd density††	Total ag mlkwds‡‡		Total milkweeds§§
1999	9267	23.00	213.2		398.5
2000	9308	19.75	183.8		370.9
2001	9186	16.92	155.4		350.4
2002	9166	14.55	133.4		324.8
2003	9267	12.49	115.8		300.4
2004	9267	10.73	99.4		277.3
2005	9247	9.21	85.2		257.2
2006	9207	7.91	72.8		240.3
2007	9247	6.79	62.8		224.2
2008	9328	5.83	54.4		201.0
2009	9389	5.00	46.9		182.6
2010	9389	4.29	40.3		167.6

*×1000; from USDA Conservation Programs (2010).
†$m^2 \times 1000$; CRP ha × 212 m^2 ha^{-1} (milkweed density from H&B, 2000) × 0.948^x (where $x = 0$ for 1999).
‡$m^2 \times 1000$; 1416 ha (Lubowski *et al.*, 2006) × 14 m^2 ha^{-1} (milkweed density from H&B, 2000) × 0.948^x (where $x = 0$ for 1999).
§$m^2 \times 1000$; 386 ha (Lubowski *et al.*, 2006) × 99 m^2 ha^{-1} (average milkweed density from H&B, 2000 and H, 2010).
¶$m^2 \times 1000$; Conservation Reserve Program (CRP) milkweeds + Pasture milkweeds + Roadside milkweeds.
** ×1000; from Iowa State Ag. Statistics (2010).
††m^2 ha^{-1}; 1999 value from H&B (2000), 2009 value from H (2010); others = 1999 value × $0.858^{x,}$ where $x = 0$ for 1999.
‡‡$m^2 \times 1000$; Ag ha × Milkweed density.
§§$m^2 \times 1000$.

acres in roadside and pasture have not changed substantially over the last decade. Data on the acres planted to corn or soybeans by year were obtained from Iowa State Agricultural Statistics (2010) and the amount of Iowa CRP land from the USDA Conservation Programs (2010).

Estimating monarch use of non-agricultural milkweeds

To estimate monarch use of non-agricultural milkweeds, we used data on the number of monarch eggs per milkweed stem from the MLMP. We examined MLMP data from 1999 to 2010 for sampling localities within the Midwest (eastern Kansas, eastern Nebraska, eastern North and South Dakota, Minnesota, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana and western Ohio). Sites were excluded in any given year if the average number of milkweeds monitored was <25 and if there were fewer than five sampling events in July and August. We also excluded garden sites because they represent a minor component of milkweeds on the landscape. Sites were excluded if volunteers observed more larvae than eggs because these volunteers may not have been able to discern monarch eggs accurately. We initially divided sites into two groups based on the habitats in which the milkweeds were found: 'natural areas' (prairies or nature preserves) and 'other' (pastures, old fields, roadsides and CRP land); there were no sites in agricultural fields. However, 'natural areas' and 'other' were not significantly different from each other in egg density and were combined in the analysis into a single 'non-agricultural' category.

For any site, the number of eggs per plant varies over the course of the season. However, there is a population build-up during July and August when the second/third generation

occurs (MLMP, 2011). We used egg density at the peak of this build-up as a metric of annual production. For each year, our estimate of production was based on the average maximum egg density over all MLMP sites. This metric does not include all of the annual production but does allow us to examine the relative differences in production among years.

Monarch use of milkweeds in agricultural fields

Pleasants monitored milkweed populations and monarch activity in agricultural fields and non-agricultural habitats in Iowa from 2000 to 2003. Initially six study sites were selected. Each site included a field planted to soybeans, another field adjacent or nearby that was planted to corn and a nearby non-agricultural habitat. Non-agricultural habitats included natural areas, pastures, old fields and roadsides. CRP land was not explicitly included as a habitat type but the non-agricultural habitats selected are similar in vegetative characteristics to CRP land. Sites were all located within a 10 km radius of Ames, Iowa, except for one site located 40 km south of Ames. Over the years of study, a few sites were removed from monitoring for logistical reasons and a few others added but in all years, both agricultural and non-agricultural plots were examined. Within each site, patches of milkweeds were marked (milkweed plots). These patches were relatively discrete units that ranged in area from 3×3 to 6×10 m and contained 10–150 milkweed stems. In each field, approximately 10 milkweed plots were chosen and mapped using a global positioning system device so they could be relocated in subsequent years. Sites were visited at weekly intervals: in 2000 from late May to late August; in 2001 from early July through late August; and in 2002 and 2003 from early June to late August. During each visit, every milkweed stem in each milkweed plot was inspected for monarch eggs and larvae.

As described above, we used the maximum number of eggs per stem observed during the weekly censuses from July through August as the measure of production. Egg densities in different non-agricultural habitat types were not statistically different, so they were combined into one category. Egg densities on milkweed in corn and soybean fields in any year were not statistically different from each other and were combined into a single category. The results are shown in Table 2. Egg densities on milkweeds in agricultural fields were significantly higher than on milkweeds in non-agricultural habitats in each year by an average factor of 3.89.

Estimating potential monarch production

Potential monarch production for any year is equal to the sum of egg production from two sources: non-agricultural and agricultural milkweeds. To calculate production from non-agricultural milkweeds, we first determined the number of milkweeds in non-agricultural habitats. This is equal to the area occupied by each habitat type (CRP land, pasture and roadside) multiplied by the density of milkweeds in that habitat (see Table 1).We then multiplied the total number of non-agricultural milkweeds by the average number of eggs per non-agricultural milkweed plant for that year from the MLMP data (see Table 3). To calculate production from agricultural fields, we first determined the number of milkweeds in fields. This is equal to the area occupied by agricultural land multiplied by the milkweed density in fields (see Table 1). The number of agricultural milkweeds in each year was multiplied by the eggs per agricultural milkweed plant. For the years 2000–2003, we used Iowa data for the eggs per agricultural milkweed (from Table 2). For each of the other years, the egg density on agricultural milkweeds was taken to be 3.89 times the MLMP value for that year (see Table 3).

Results

Estimates of milkweed numbers on the Iowa landscape (Table 1) show that milkweeds declined in both agricultural fields and non-agricultural habitats from 1999 to 2010. There was a 31% decline for non-agricultural milkweeds and an 81% decline for agricultural milkweeds with a 58% overall decline for total milkweeds. In 1999, milkweeds in agricultural fields constituted 53% of total milkweeds, but by 2010 were only 24% of the total. The 58% loss of milkweeds on the landscape actually underestimates the loss of resource for monarchs, because most

Table 2. Maximum eggs per milkweed stem July through August for agricultural and non-agricultural sites in Iowa where '*n*' is the number of fields examined. Egg densities on milkweeds in agricultural fields were significantly higher than on milkweeds in non-agricultural habitats in each year (2000: $t = 3.97$, d.f. = 11; 2001: $t = 2.90$, d.f. = 4; 2002: $t = 3.35$, d.f. = 4: $t = 4.54$, d.f. = 5; all P-values < 0.02).

	Maximum eggs per milkweed						
	Agricultural			Non-agricultural			
Year	Avg.	SE	*n*	Avg.	SE	*n*	Ratio ag/non-ag
2000	0.796	0.140	10	0.197	0.049	8	4.05
2001	1.661	0.459	5	0.329	0.021	3	5.05
2002	0.659	0.123	4	0.205	0.056	4	3.21
2003	1.125	0.108	5	0.345	0.133	3	3.26
						Average ratio	3.89

Table 3. Estimate of egg production in the Midwest from 1999 to 2010. Note that values in the final three columns are relative; egg densities are in eggs/stem whereas milkweed densities are not in stems ha^{-1} but m^2 ha^{-1}.

Year	Total non-ag milkweeds*	Total ag milkweeds*	Eggs/ plant-non-ag†	Eggs/ plant-ag‡	Production non-ag§	Production ag¶	Total production**
1999	185.4	213.2	0.243	0.945	45.0	201.4	246.5
2000	187.1	183.8	0.144	0.796	26.9	146.3	173.2
2001	195.0	155.4	0.299	1.661	58.3	258.2	316.5
2002	191.5	133.4	0.197	0.659	37.6	87.9	125.5
2003	184.7	115.8	0.173	1.125	31.9	130.2	162.1
2004	177.9	99.4	0.102	0.395	18.1	39.3	57.4
2005	172.0	85.2	0.205	0.796	35.2	67.8	103.0
2006	167.5	72.8	0.277	1.077	46.4	78.5	124.9
2007	161.4	62.8	0.274	1.066	44.2	66.9	111.1
2008	146.6	54.4	0.154	0.599	22.6	32.6	55.2
2009	135.7	46.9	0.120	0.465	16.2	21.8	38.0
2010	127.4	40.3	0.311	1.210	39.6	48.7	88.4

*$m^2 \times 1000$; from Table 1.
†from MLMP.
‡Non-ag eggs/plant × 3.89 (ratio of ag to non-ag, see Table 2), except for 2000–2003 from Table 2.
§Total non-ag milkweeds × Eggs/plant non-ag.
¶Total ag milkweeds × Eggs/plant ag.
**Production non-ag. + Production ag.

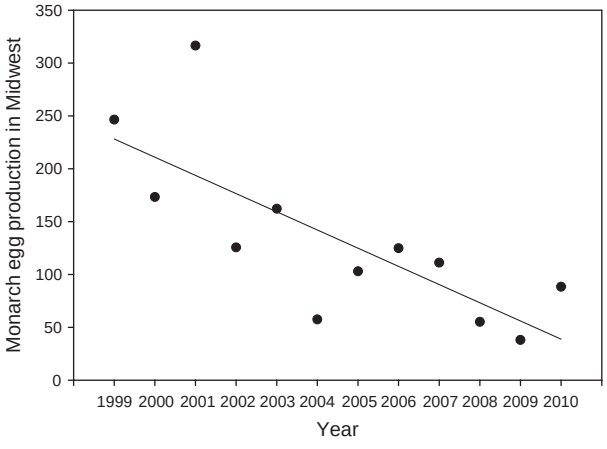

Fig. 2. Estimate of monarch production from the Midwest by year. Linear regression $F_{1,11} = 13.7$; $P = 0.004$; $r^2 = 0.58$.

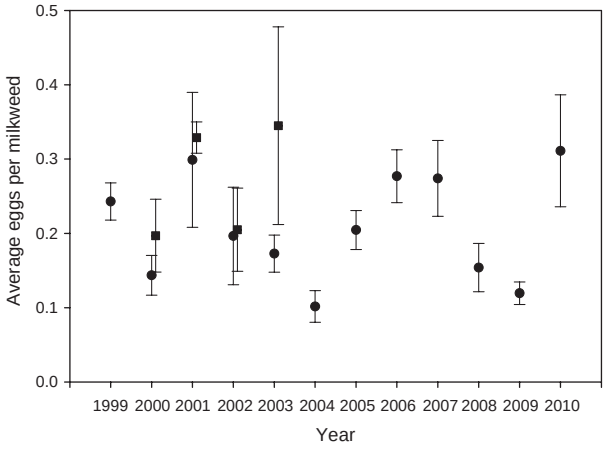

Fig. 3. Average maximum egg density (eggs per milkweed stem ± 1 SE) for July through August for non-agricultural milkweeds at sites throughout the Midwest (from MLMP). Number of sites: 1999, 16; 2000, 41; 2001, 13; 2002, 25; 2003, 41; 2004, 46; 2005, 49; 2006, 57; 2007, 29; 2008, 29; 2009, 30; 2010, 21. Square symbols indicate the average value (±1 SE) for non-agricultural sites in Iowa (from Table 2). The Iowa value for each year was not significantly different from the MLMP value.

of the loss was in agricultural fields and each agricultural milkweed represents 3.89 times more monarch eggs than a non-agricultural milkweed (Table 2). If the numbers of agricultural milkweeds in Table 1 are multiplied by 3.89 to convert them to their resource potential, the decline in the milkweed resource base is 72%. Of this potential resource lost, 92% comes from agricultural fields and 8% from non-agricultural habitats. Table 3 shows the conversion of yearly milkweed numbers into monarch production. The relative contribution of agricultural milkweeds to total monarch production went from 82% in 1999 to 55% in 2010.

There has been a significant decline in monarch egg production over the last decade (Fig. 2 – linear regression $F_{1,11} = 13.7$, $P = 0.004$, $r^2 = 0.58$). On the basis of regression equation for this decline ($y = 254.4 - 17.21x$, where $x = 1$ when the year is 1999), we estimate that between 1999 and 2010 monarch egg production in the Midwest was reduced 81%. This decline in production would not have occurred if monarchs had increased their use of the remaining milkweeds as agricultural milkweeds declined. However, egg density on non-agricultural milkweeds from the MLMP data did not show a significant change over the years (Fig. 3) (because of non-normality, a Poisson regression was used; Wald $\chi^2 = 0.15$; d.f. = 1; $n = 398$, NS). We

also compared our estimate of potential monarch production in each summer with the size of the population that subsequently overwintered in Mexico (Fig. 4). Yearly production values were positively correlated with the size of the overwintering population (linear regression $F_{1,11} = 8.97$, $P = 0.01$, $r^2 = 0.47$).

Discussion

Our estimate of monarch production decline in the Midwest was based in part on Iowa data. To what extent do Iowa data reflect the Midwest as a whole? We used Iowa data to estimate (i) the proportion of milkweed in various habitats, (ii) the density of milkweeds in each habitat, (iii) the decline in milkweeds in agricultural fields and (iv) the relatively higher egg density on agricultural milkweeds compared to non-agricultural milkweeds. We examine each of these aspects of the data. (i) Data on land use for the Midwestern states (Lubowski *et al.*, 2006) show that of the potential milkweed habitat 73% was in crop production and 27% in non-agricultural habitats (6% in CRP land, 6% in cropland pastures, 11% in grassland and range pastures, and 4% in roadsides). This is similar to the 79% in crop production for the state of Iowa and 21% in non-agricultural habitats (6% in CRP land, 5% in cropland pastures, 7% in grassland and range pastures and 3% in roadsides). Note that these values do not include forested land as this is not milkweed habitat. This comparison excluded the Northern Plains states (Kansas, Nebraska, N. and S. Dakota), which have extensive grasslands and rangeland in the western sections. If those states are included, the per cent of Midwest land in crops falls to 60% with 40% of land non-agricultural. (ii) Iowa data were used to estimate milkweed densities for agricultural and roadside habitats; the change in milkweed density in other non-agricultural habitats was based on Midwest MLMP data. There has not been a long-term study of milkweed density in agricultural habitats outside of Iowa so the similarity between Iowa and the Midwest in this aspect can only be assumed. (iii) Other Midwest areas have seen a decline in milkweed density in agricultural fields over the past decade. Two of the Wisconsin fields originally surveyed by Oberhauser in 2000 (Oberhauser *et al.*, 2001) were resurveyed in subsequent years. In 2000, these sites had an average of 0.28 milkweed stems m^{-2}, and in 2002–2006, after the growers began to use glyphosate-tolerant soybeans in 2001, no milkweeds were found. (iv) Higher egg densities on agricultural milkweeds were also observed in other states in the Midwest in 2000 (Oberhauser *et al.*, 2001).

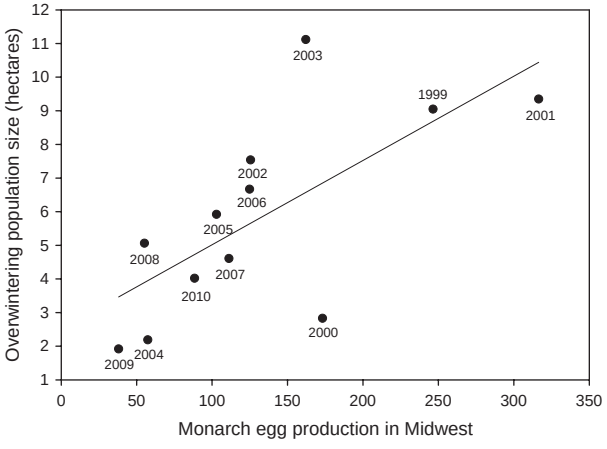

Fig. 4. Estimate of monarch production in the Midwest by year (from Table 3) compared with the size of the subsequent overwintering population in that year (from Rendón-Salinas et al., 2011). The size of the overwintering population is measured in hectares covered by butterflies. Linear regression $F_{1,11} = 8.97$; $P = 0.01$; $r^2 = 0.47$.

Further evidence suggesting that our approach, which combines data from Iowa and Midwest sources, does reflect production for the Midwest as a whole comes from the significant positive correlation between the annual estimate of monarch production and the size of the subsequent overwintering population (Fig. 4). Because the Midwest contributes about half of the individuals to the overwintering population (Wassenaar & Hobson, 1998), we would expect such a correlation if Midwest production were accurately estimated. We note, however, that the estimate of the Midwest contribution to the overwintering population was made before significant glyphosate use in row crops and only represents 1 year of data.

Although our estimates of annual Midwest monarch production were highly correlated with the size of the subsequent overwintering population, these estimates explained only 47% of the variation in the size of the overwintering population. In particular, our production value for 2003 underestimated the overwintering population size and our value for 2000 overestimated it. We suggest four possible reasons for such deviations. (i) Deviations may be due to the fact that we have used egg density as our measure of production, which is a measure of potential production, while actual production is adult butterflies. The relationship between potential and actual production will depend on survivorship from egg to adult, which may vary among years (J. M. Pleasants & K. S. Oberhauser, unpubl. data). (ii) The relative contribution of the Midwest to the population as a whole is likely to vary from year to year (K. S. Oberhauser, unpubl. data). (iii) The amount of mortality during the fall migration is likely to vary among years depending on conditions along the migratory route including nectar availability, temperature, weather events, drought conditions and wind conditions. (iv) We used a factor of 3.89, the average of 4 years of Iowa data, to convert agricultural milkweeds into their monarch egg production. The factor varies among years, as seen in Table 2, and may be somewhat different in other areas of the Midwest.

The differences between years in egg density per stem seen in the MLMP data (Fig. 3) are likely to be caused by factors in addition to the effect of resource availability. The MLMP egg densities we used came from the second and third generation of monarchs. The size of each generation will depend on the size of the previous generation, each of which will be influenced by the prevailing weather conditions during egg laying and larval development (Zalucki & Rochester, 2004). Although the overwintering population begins this sequence, we found no correlation between the size of the overwintering population and monarch production the following summer. This indicates that other factors, principally temperature and weather conditions, can erase

the influence of the starting population. But environmental conditions alone do not govern population size. Even if favourable conditions exist, the potential production of the monarch population is dampened by the loss of milkweeds.

As previously mentioned, the loss of milkweeds in agricultural fields would not have affected total monarch production if monarch use of the remaining milkweeds, both agricultural and non-agricultural, had increased sufficiently. We do not have data on the use of agricultural milkweeds over the last decade but data from the MLMP indicate that there was no increase in use of non-agricultural milkweeds over this period (Fig. 3). In a modelling study, Zalucki and Lammers (2010) found that removing small patches of milkweed from the matrix (the area between larger patches of milkweed) made it harder for monarch females to achieve their egg production potential because of increased search time. In their model, a decrease in milkweed availability in the agricultural crop matrix, such as what would result from herbicide use, could significantly reduce the lifetime number of eggs laid by individual females.

Davis (2011) has suggested that there has been no downward trend in monarch production, based on monitoring data at two sites at which monarchs congregate during the fall migration. The monarchs that appear at these two sites, Peninsula Point in Upper Michigan and Cape May in New Jersey, are migrants from the Upper Peninsula of Michigan and south central Canada, and the Eastern United States, respectively. However, the isotope analysis of (Wassenaar and Hobson (1998) indicates that monarchs from these areas constitute a much smaller portion of the total monarch population than monarchs from the Midwest. Consequently, the lack of decline Davis observed will not reflect the population as a whole. Similar points have also been argued by Brower *et al.* (2011a).

The lack of decline in migrating Eastern monarchs, noted by Davis, further illustrates the connection between glyphosate use in corn and soybean fields and monarch decline. Monarchs from the Northeast and Canada may not be experiencing a decline because they come from areas with less corn and soybean agriculture and thus less milkweed loss because of herbicide use. In 2010, there were 25.1 million soybean hectares and 25.5 million corn hectares in the Midwest but only 0.4 million soybean hectares and 0.7 corn hectares in the Northeast (USDA, National Agricultural Statistics Service, 2011c).

We estimated that monarch production in the Midwest had declined 81% from 1999 to 2010. For comparison, there was a 65% decline in the size of the overwintering population over this same period (Brower *et al.*, 2011b). The similarity of these percentages, and the fact that our estimate of Midwest production is strongly correlated with the size of the overwintering population, clearly show the dominance of Midwest production for the population as a whole. However, the fact that the size of the overwintering population has declined less than the population contribution from the Midwest reflects the mitigating effect of portions of the range of the species that are not dominated by corn and soybean agriculture and have not been impacted by milkweed loss. As the monarch production contribution from the Midwest declines, the relative contribution from other parts of the range increases. A reassessment of the production contribution of the Midwest and other parts of the range, such as that performed earlier by Wassenaar and Hobson (1998), would be useful.

The loss of milkweeds in agricultural fields is particularly devastating for the monarch population because agricultural milkweeds are more heavily used than non-agricultural milkweeds (Table 2). This difference in egg density could result if females that find patches of milkweeds in agricultural fields lay more eggs per stem or if more females find patches of agricultural milkweeds. Patch size is typically smaller in agricultural fields than in non-agricultural habitats (J. M. Pleasants & K. S. Oberhauser, pers. obs.), and higher egg densities per stem are observed in smaller milkweed patches (Zalucki & Suzuki, 1987). Monarch females may seek out smaller patches and oviposit more heavily there, perhaps because small patches tend to support greater larval success (Zalucki, 1981; Zalucki & Kitching, 1982). Greater oviposition by individual females may also be due to their perception of agricultural milkweeds as being of higher quality. Agricultural milkweed leaves have higher nitrogen content (J. M. Pleasants, unpubl. data) and tend to be in better condition. Finally, the milkweed chemical signal that attracts monarch females may be more apparent against the monoculture background of agricultural fields making it easier for females to find milkweeds in this habitat.

One unexpected finding in this study was the decline in milkweed density in non-agricultural habitats based on measurements by MLMP volunteers. These patches were not chosen at random, and it is possible that this decline is not representative of milkweeds in non-agricultural habitats across the landscape. Milkweed is a disturbance species and as such we would expect colonisation of disturbed areas followed by a population increase for a number of years and then a population decline as milkweed is outcompeted by later successional species. Monitored patches were chosen because they contained high milkweed densities. Thus, they may represent populations that had already experienced some growth and were now in the declining phase. A more thorough survey of milkweed densities in randomly chosen non-agricultural habitats over time is needed. If milkweed densities in non-agricultural habitats are not declining, then the loss of monarch production is not as large as we have estimated. We reran our calculations assuming no decline, and the estimated loss of monarch production from 1999 to 2010 was 76%, somewhat lower than the 81% decline estimated using decreasing milkweed densities in non-agricultural habitats.

Given the disappearance of milkweeds in agricultural fields, milkweeds present in other habitats become more important for monarch populations. Table 1 indicates that the habitat of greatest importance is CRP land. However, the amount of CRP land is also declining; in 2010, the number of CRP hectares for the Midwestern states had declined by 0.5 million from its high in 2007 of 3.8 million hectares (USDA, Conservation Programs, 2010). Farmers have a number of options with regard to what types of vegetation to use as cover on CRP land, with grasses predominating. Adding forbs, including milkweeds, to planting mixes would provide nectar sources that could benefit many insect species and provide host plants for monarchs. While persuading farmers to include milkweed seed in the mix may be difficult, milkweed is capable of colonising such habitats on its own

and education efforts about the value of milkweed and the many non-weedy milkweed species available are underway (Monarch Joint Venture, 2011). Further research needs to be undertaken on CRP land to see how different types of cover vegetation and land management practices affect milkweeds and monarchs.

Roadsides can provide important milkweed habitat; in 2010, 20% of the milkweeds were in roadsides (Table 1), and this value will increase as the remaining agricultural milkweeds disappear. The treatment of roadsides by departments of transportation could influence their value to monarchs. Roadsides are often mowed and sprayed with herbicides to eliminate forbs but roadside management plans compatible with monarch conservation could be developed. Many states are implementing programmes to plant native species along roadsides; such programmes could consider adding milkweeds.

We have not yet seen the full impact that the use of glyphosate herbicides and the consequent reduction in milkweed resources will have on the monarch population. At present, some milkweeds still remain in agricultural fields. Given the established dominance of glyphosate-tolerant crop plants and widespread use of glyphosate herbicide, the virtual disappearance of milkweeds from agricultural fields is inevitable. Thus, the resource base for monarchs in the Midwest will be permanently reduced. This will set a new, lower ceiling for monarch population size. A lower population size could lead to greater vulnerability of the population to deforestation on the overwintering sites and to extreme weather events or climate changes on the overwintering sites, in breeding areas and along migratory routes (Brower *et al.*, 2011b).

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Author contributions

J.P. performed field studies in Iowa, analysed the data and was primarily responsible for writing the article. K.O. supervised the collection and collation of the data from the MLMP and participated in the writing of the article.

Competing financial interests

The authors declare no competing financial interests.

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Hansen
7 Feb 2013

Reasons for Labeling of Genetically Engineered Foods

March 19, 2012

TO: AMA Council on Science and Public Health

FROM: Michael Hansen, Ph.D., Senior Scientist, Consumer Reports

RE: Resolutions 508 (Illinois) and 509 (Indiana) Supporting Federal Legislation and/or Regulations that Require Clearly Labeling Food with Genetically Engineered Ingredients

SUMMARY: Based on the scientific uncertainty surrounding both the molecular characterization of genetically engineered (GE) crops as well as the detection of potential allergenicity, there is more than enough uncertainty to decide to require labeling of foods produced via GE as a risk management measure as a way to identify unintended health effects that may occur post approval. If foods are not labeled as to GE status, it would be very difficult to even identify an unexpected health effect resulting from a GE food.

Dear Council Members:

I am writing to submit scientific evidence which strongly supports the intent of *Resolutions 508 and 509 Supporting Federal Legislation and/or Regulations that Require Clearly Labeling Food with Genetically Engineered Ingredients.* Consumer Union[1] supports mandatory labeling for foods produced with genetically engineered (GE) ingredients for a number of reasons.

1. **There has been global agreement that genetically engineered foods are different than conventionally bred foods and that all genetically engineered foods should be required to go through a safety assessment prior to approval.** Codex Alimentarius is the food safety standards organization of the United Nations, and is jointly run by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). From 2000 – 2008, there were two rounds of the Codex Alimentarius *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology. This Task Force developed a number of documents, including a Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45, 2003)[2]; there are separate Guidelines for GE animals and GE microorganisms, as well. The World Trade Organization (WTO) considers that, in terms of food safety, the standards or guidelines of Codex Alimentarius are deemed the global science-based standard and, thus, immune to trade challenges, i.e. they are not considered to be a "non-tariff trade barrier."

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[1] *Consumers Union is the public policy and advocacy division of Consumer Reports. Consumers Union works for telecommunications reform, health reform, food and product safety, financial reform, and other consumer issues. Consumer Reports is the world's largest independent product-testing organization. Using its more than 50 labs, auto test center, and survey research center, the nonprofit rates thousands of products and services annually. Founded in 1936, Consumer Reports has over 8 million subscribers to its magazine, website, and other publications, and a few noncommercial grants. Roughly 8 million people subscribe to Consumer Reports or Consumer Reports online.*

[2] At: http://www.codexalimentarius.net/web/standard_list.do?lang=en

The reason for two rounds of the Codex Alimentarius *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology came as a result of a global agreement that genetic engineering is a process that is sufficiently different from conventional breeding that foods developed via genetic engineering should go through a safety assessment before such foods are allowed on the market. For information on the ways genetic engineering differs from conventional breeding, see Hansen, 2000.[3]

Last year, after more than 15 years of debate, the Codex Committee on Food Labeling agreed to forward a document on labeling of GE foods to the Codex Alimentarius Commission for approval. Last July, at the conclusion of the meeting of the Codex Alimentarius Commission, the World Health Organization News put out a letter to journalists, noting that the "Codex Alimentarius Commission has stated that governments are free to decide on whether and how to label foods derived from modern biotechnology, including foods containing genetically-modified organisms. The labeling should be done in conformity with the text approved by the Codex Commission, to avoid a potential trade barrier. The decision, which will help inform consumers' choices regarding genetically-modified foodstuffs, was taken at the 34th Session of the Commission, held in Geneva from 4-9 July 2011. More than 600 delegates from 145 of the 184 member countries, UN, inter-governmental and non-governmental organizations attended."[4]

Unlike all other developed countries, the US Food and Drug Administration (FDA) does not require safety testing for GE plants. The FDA's original policy on GE (or GM, for genetically modified) plants was introduced at a press conference at an industry gathering on May 28, 1992 by then Vice-President Dan Quayle as a de-regulatory initiative. The policy was based on the notion "that the new techniques [e.g. genetic engineering] are extensions at the molecular level of traditional methods and will be used to achieve the same goals as pursued with traditional plant breeding,"[5] and therefore should be regulated in the same way. In other words, no requirement for human safety testing; instead there are "voluntary safety consultations."

The lack of adequate safety testing can be seen in the letter FDA sends to the company after completion of a "safety consultation." For example, the letter sent to Monsanto on September 25, 1996 about one of their first Bt-corn varieties, MON810, states, "Based on the safety and nutritional assessment you have conducted, **it is our understanding that Monsanto has concluded that corn grain and forage derived from the new variety are not materially different in composition, safety, or other relevant parameters from corn grain and forage currently on the market, and that they do not raise issues that would require premarket review or approval by FDA" bold** added.[6] Note that FDA does not state its own opinion about

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[3] Hansen, M. 2000. Genetic engineering is not an extension of conventional plant breeding: How genetic engineering differs from conventional breeding, hybridization, wide crosses and horizontal gene transfer. 13 pp. At: http://www.consumersunion.org/food/widecpi200.htm

[4] Email from WorldHealthOrganizationNews@who.int to journalists dated July 9, 2011.

[5] Pg. 22991 in FDA. Statement of Policy: Foods Derived From New Plant Varieties, May 29, 1992, Federal Register vol. 57, No. 104. At: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096095.htm

[6] At: http://www.fda.gov/Food/Biotechnology/Submissions/ucm161107.htm

the safety of this crop; it only states what the company believes. The letters for all 84 "safety consultations" done since the Flavr Savr tomato contain basically the same language. This clearly shows that the FDA does not conduct safety assessments.

Other scientists have noted the lack of proper safety testing. For example, Dr. Belinda Martineau, the scientist who conducted the safety studies on the first GE plant, the Flavr Savr tomato (engineered for long shelf life) at Calgene, points out in her book **First Fruit: the Creation of the Flavr Savr Tomato and the Birth of Biotech Foods:** "Rather than personal opinion, the scientific community should give the public facts, hard facts; the results of studies that indicate these foods are safe to eat and that growing them on a large scale will not cause environmental damage. Scientists and regulators throughout the ag biotech industry agree that more public education about genetic engineering research is necessary, but, thus far, few have provided much information beyond how the technology works and the wondrous things that might be done with it. . . . *And simply proclaiming that 'these foods are safe and there is no scientific evidence to the contrary' is not the same as saying 'extensive tests have been conducted and here are the results.' In fact, without further elaboration, 'no scientific evidence to the contrary' could be construed as 'no scientific evidence, period.'* "[7] italics added.

Since the 1992 Statement of Policy on genetically engineered food, FDA has admitted that its original policy was based on a false notion. In 2001, the FDA proposed requiring companies to notify the government at least 120 days before commercializing a transgenic plant variety. As part of that proposed rule, the FDA admits that insertional mutagenesis is a problem and suggests requiring data on each separate transformation event: "[B]ecause some rDNA-induced unintended changes are specific to a transformational event (e.g. those resulting from insertional mutagenesis), FDA believes that it needs to be provided with information about foods from all separate transformational events, even when the agency has been provided with information about foods from rDNA-modified plants with the same intended trait and has had no questions about such foods. *In contrast, the agency does not believe that it needs to receive information about foods from plants derived through narrow crosses* [e.g. traditional breeding]" italics added (FR 66(12), pg. 4711).[8] In other words, FDA has admitted that there is a difference between GE and traditional breeding. In spite of this, FDA is still following the 1992 policy rather than the 2001 policy.

Global agreement has been reached on what constitutes proper safety assessment of foods derived from GE plants, yet such suggested studies have not been carried out on GE Bt corn (or any other GE crop approved in the US). In 2003, the Codex Alimentarius *Ad Hoc* Task Force on Foods Derived from Biotechnology reached agreement on a "Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants."[9] This Guideline was formally adopted by the full Codex Alimentarius Commission in 2003, and was updated in 2008. This is important because in the case of trade disputes, the World Trade Organization considers

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[7] Pp. 232-233 in Martineau, B. 2001. First Fruit. McGraw-Hill.

[8] Pg. 4711 in FDA. Premarket Notice Concerning Bioengineered Foods. Federal Register January 18, 2001. Federal Register Vol. 51(12): pp. 4706 – 4738. At: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096149.htm

[9] See Codex Alimentarius Guideline 45. At: http://www.codexalimentarius.net/web/standard_list.do?lang=en

that, in terms of food safety, the standards or guidelines of Codex Alimentarius are deemed the global science-based standard and, thus, immune to trade challenges, i.e. they are not considered to be a "non-tariff trade barrier." At present, none of the GE plants on sale in the US can meet this standard.

Since the US does not require safety assessments of GE plants, while the Codex Alimentarius Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants states that such a food safety assessment should be done, this means the US cannot meet the global standards for safety assessment of GE foods. Consequently, countries that require food safety assessments for GE foods could block shipments of such GE foods from the US without fear of losing a WTO challenge.

We believe that the US should require safety assessments on foods derived from GE organisms, and that those safety assessments should be consistent with the guidelines developed by the Codex Alimentarius *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology so that US food products are not potentially subject to a WTO challenge from another country.

2. **Significant scientific uncertainty exists in the risk analysis of foods derived from GE and this is recognized in the Codex.** In fact, the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants has a whole section on unintended effects which clearly states that they can have an unintended effect on human health: *"Unintended effects due to genetic modification may be subdivided into two groups: those that are "predictable" and those that are "unexpected" . . . A variety of data and information are necessary to assess unintended effects because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health."*[10] italics added (paras 16 and 17, CAG/GL 45-2003). Furthermore, this section recognizes that the unintended effects could also be caused by changes in genes that are expressed at the molecular level and how the gene products are processed: "Molecular biological and biochemical techniques (that) can also be used to analyze potential changes at the level of gene transcription and message translation that could lead to unintended effects" (para 16, CAG/GL 45-2003).

3. **Labeling of GE food can serve as a risk management measure to deal with scientific uncertainty.** This would be consistent with the recommendations developed by the Codex Alimentarius Ad Hoc Intergovernmental Task Force on Foods Derived from Modern Biotechnology and adopted by the Codex Alimentarius Commission in 2003. The *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology* (CAC/GL 44—2003) clearly state that labeling can be used as a risk management option to deal with scientific uncertainties associated with the risk assessment of GE foods: "18. Risk managers should take into account the uncertainties in the risk assessment and implement appropriate measures to manage these uncertainties. 19. Risk management

[10] pars 18, 19 in CAC/GL 44—2003. At: http://www.codexalimentarius.net/web/standard_list.do?lang=en

measures may include, as appropriate, food labeling, conditions for market approval and post-market monitoring."[11]

If there are unexpected adverse health effects that happen as a result of GE, then labeling could serve as a risk management mechanism that would allow us to track such health problems if they arose. If a food with GE ingredients is not labeled as such, and that food causes an adverse health effect, such as an allergic reaction, there would be virtually no way to determine that the GE process was linked to the adverse health effect. For example, suppose a company decides to insert a synthetic gene, which codes for a modified protein, into tomatoes. Suppose that the novel protein causes a strong but delayed (say by 24 hours) allergic reaction (e.g. serious rash, upset stomach, or anaphylactic shock) in some relatively small subset of the population. To start with, doctors would have an extremely difficult time identifying the source of the problem. If the offending tomato variety is not very prevalent (i.e. does not have a large market share), then the regular allergy test, making a list of all foods eaten in the last 24 hours, might not uncover the tomato as the source of the problem (the person would have to obtain and eat the offending tomato variety a second time and get the same reaction). It might well take large numbers of people being adversely affected and having the offending tomato variety be a large share of the market before there would be any hope of figuring out what was causing the problem.

Even if the food *has* undergone rigorous premarket safety testing, scientific uncertainties remain associated with the risk analysis. In addition, when a large population (in the millions or tens of millions) is exposed to a GE food, rare unexpected health problems can appear. Take the case of Vioxx, a drug that was found to be safe in premarket testing but had to be removed from the market after adverse health effects were seen when the drug was used by large numbers of people. Because these drugs are labeled, doctors are able to associate the unexpected health problem with the specific drugs. With GE foods, labeling would serve a similar purpose.

In addition to FDA not requiring any premarket safety testing, there is virtually no independent safety testing of these crops in the US due to intellectual property rights. When farmers buy GE seed in the US, they invariably must sign a product stewardship agreement which forbids them from giving such seeds to researchers.[12] In addition, researchers must get permission from the biotech companies before they can do research, which means there is a paucity of independent research. Scientists have even been threatened with legal action if they revealed information obtained via freedom-of-information.[13] In early 2009 26 public sector scientists in the US took the unprecedented step of writing to the US Environmental Protection Agency (EPA) protesting that "as a result of restricted access, no truly independent research can be legally conducted on many critical questions regarding the technology."[14] As a result, the editors of *Scientific American* published a perspective stating that "we also believe food safety and environmental protection depend on making plant products available to regular scientific scrutiny. Agricultural technology companies should therefore immediately remove the restriction

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[11] At: http://www.codexalimentarius.net/web/standard_list.do?lang=en

[12] Waltz, E. 2009. Under wraps. *Nature Biotechnology*, 27(10): 880-882. At: http://www.emilywaltz.com/Biotech_crop_research_restrictions_Oct_2009.pdf

[13] IBID

[14] http://www.scientificamerican.com/article.cfm?id=do-seed-companies-control-gm-crop-research

on research from their end-user agreements." We concur and believe that only truly independent safety tests will give us an answer about the safety of GE foods. In the meantime, it's crucial that GE foods be labeled as a risk management measure to deal with scientific uncertainty.

4. **We believe that consumers have a right to know what is in the food they eat.** A number of polls from 1995 to 2011 have found that between 70% and 95% of people polled supported mandatory labeling.[15] "Information of material importance" to consumers is far broader than just "changes in the organoleptic, nutritional or functional properties" of a food. The fact that more than 850,000 people have sent comments to the FDA in support of a citizen's petition asking FDA to require labeling of GE foods, shows that consumers overwhelmingly want food from GE sources to be labeled as such.[16] In addition, on March 12, 2012, US Senator Barbara Boxer and Congressman Peter DeFazio joined with 53 other Senate and House lawmakers in sending a letter urging the FDA to require the labeling of GE foods.[17]

FDA has tried to argue that they don't have the authority to label GE foods unless there is a "material change" in the food, which FDA defines as "change in the organoleptic, nutritional or functional properties" of the food that is not obvious to the consumer at the point of purchase. We strongly disagree with FDA and feel that they are trying to ignore their own history. In the past FDA has required labeling under the "material fact" analysis that did not entail a change in nutritional value, organoleptic properties, or functional characteristics of a food. FDA's authority to require labeling of all foods derives, in part from section 201(n) and 403(a)(1) of the Federal Food Drug and Cosmetic Act. A label is considered "misleading" if it "fails to reveal facts that are **material** in light of representations made. . ." **bold** added. FDA articulated this position in the 1986 final rule that required labeling of irradiated foods, even though the FDA had ruled that irradiated foods were safe. FDA stated in this final rule on food irradiation that the large number of respondents who asked for labeling of retail products was one factor indicative of the materiality of food irradiation: "***Whether information is material under section 201(n) of the act depends not on the abstract worth of the information but on whether consumers view such information as important and whether the omission of label information may mislead a consumer. The large number of consumer comments requesting retail labeling attest to the significance placed on such labeling by consumers***"[18] emphasis added. **Thus, materiality clearly does not always include "some change in nutritional value, organoleptic properties, or functional characteristics"** of the food.

Material facts other than material changes have long been required for other reasons that are important to consumers. Labeling the source of protein hydrolysates was required because of the concern of vegetarians and observant Jews and Muslims. As the FDA stated, "the food source of a protein hydrolysate is information of material importance for a person who desires to avoid certain foods for religious or cultural reasons."[19] Thus, "information of material

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[15] http://gefoodlabels.org/gmo-labeling/polls-on-gmo-labeling/
[16] http://gefoodlabels.org/
[17] IBID
[18] Pg. 13380. FDA. Final Rule on Food Irradiation. Federal Register April 18, 1986, Federal Register, Vol. 51, pg. 13376
[19] 56 FR 28592 [1991]

importance" to a consumer is not simply restricted to "information about the characteristics of a food."

In 2007, FDA proposed a revision to their labeling requirements for irradiated foods, such that labeling would only be required on those irradiated foods in which the irradiation has lead to a "material change"—defined as a "change in the organoleptic, nutritional or functional properties"—in the food that is not obvious to the consumer at the point of purchase. Thus, not all irradiated food would be required to be labeled. This proposed revision to the irradiation labeling standard went nowhere. However, this attempted weakening of the food irradiation labeling standard clearly demonstrates that FDA is now trying to narrow the concept of "materiality," so as to avoid the labeling of GE foods.

A number of recent scientific studies have pointed out unexpected effects in genetically engineered crops and have shown that they can lead to potential adverse health effects:

- **GE plant materials are finding their way into the human body.** A study done by Canadian scientists and published last year was very disturbing. The study involved 30 pregnant and 39 non-pregnant women in Quebec, Canada.[20] Blood was taken from women and from fetal cord blood and tested for 3 pesticides associated with GM: glyphosate, glufosinate, and Cry1Ab. The surprising finding was that Cry1Ab was detected in 93% and 80% of maternal and fetal blood samples, respectively and in 69% of tested blood samples from nonpregnant women. The scientists noted that "trace amounts of the Cry1Ab toxin were detected in the gastrointestinal contents of livestock fed on GM corn, raising concerns about this toxin in insect-resistant GM crops; [suggesting] (1) that these toxins may not be effectively eliminated in humans and (2) there may be a high risk of exposure through consumption of contaminated meat."[21] They concluded, "To our knowledge, this is the first study to highlight the presence of pesticides-associated genetically modified foods in maternal, fetal and nonpregnant women's blood. 3-MPPA and Cry1Ab toxins are clearly detectable and appear to cross the placenta to the fetus. Given the potential toxicity of these environmental pollutants and the fragility of the fetus, more studies are needed, particularly those using the placental transfer approach."[22]

- **A major food safety concern for GE plants is allergenicity.** In 2001, the report of a Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation on Allergenicity of Foods Derived from Biotechnology, held at WHO headquarters in Rome, laid out a detailed protocol (a decision tree) for evaluating the allergenicity of GE foods.[23] None of the GE

[20] Aris, A and S Leblanc. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reproductive Toxicology*, 31(4): 528-533.
[21] Pg. 533 in Aris, A and S Leblanc. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reproductive Toxicology*, 31(4): 528-533.
[22] IBID
[23] FAO/WHO. 2001. Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, January 22-25, 2001. Rome, Italy. At: ftp://ftp.fao.org/es/esn/food/allergygm.pdf

crops, including GE corn, on the market in the U.S. have been assessed using such a protocol.

- **Various types of scientific evidence suggest that Bt corn may contain a transgenic allergen.** Bt corn contains various modified endotoxins from the soil bacterium *Bacillus thuringiensis* (Bt). These δ-endotoxins are called Cry proteins, in particular Cry1Ab or Cry1Ac. A study of farmworkers who worked in onion fields where foliar Bt sprays were used found that 2 of them contained antibodies to the δ-endotoxins, Cry1Ab and/or Cry1Ac, consistent with an allergy.[24] A survey of Bt cotton farmers in India done by local doctors found that numerous Bt cotton farmers, as well as workers in a ginning factory, had symptoms consistent with an allergic reaction to Bt cotton within a year of the introduction of Bt cotton in the region.[25]

- **One of the endotoxins found in GE corn, Cry1Ac, has been found to have sequence similarity to a known human allergen.** One of the first steps in assessing the allergic potential of a protein (most allergens are proteins) is to determine if it has similarity in amino acid sequence to a known allergen. A paper published in 1998 by the head of FDA's own biotechnology studies branch, Dr. Steven Gendel, found significant amino acid sequence similarity between Cry1Ab and Cry1Ac (found in Bt maize and Bt cotton) and vitellogenin, the main precursor to egg yolk protein and a known allergen, as well as between Cry3A (Bt potatoes) and β-lactoglobulin, a major milk allergen.[26]

- **Scientific studies also show Cry1Ac has a strong effect on the immune system as well as being a potent adjuvant.** A series of five studies carried out by a team of scientists from two Mexican universities and from Cuba have suggested that the Cry1Ac protein has immunogenic and allergenic properties. A mouse study demonstrated that the Cry1Ac was a potent systemic and mucosal adjuvant: "We conclude that Cry1Ac is a mucosal and systemic adjuvant as potent as CT [cholera toxin] which enhances mostly serum and intestinal IgG antibody responses".[27] Another mouse study which further characterized the mucosal and systemic immune response induced in mice "confirm[ed] that the Cry1Ac protoxin is a potent immunogen able to induce a specific immune response in the mucosal tissue, *which has not been observed in response to most other proteins*"

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[24] Bernstein, I.L., Bernstein, J.A., Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z., Selgrade, M.K., Doerfler, D.L. and V.L. Seligy. 1999. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environmental Health Perspectives*, 107(7): 575-582. At: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1566654/pdf/envhper00512-0103.pdf

[25] Gupta, A. et al. 2006. Impact of Bt cotton on farmers' health (in Barwani and Dhar District of Madhya Pradesh). At: http://www.lobbywatch.org/archive2.asp?arcid=6265 and http://www.lobbywatch.org/archive2.asp?arcid=6266

[26] Gendel, S.M. 1998. The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Advances in Food and Nutrition Research*, 42: 44-61.

[27] Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., de la Riva, G.A. and R. Lopez-Revilla. 1999. Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. *Scandinavian Journal of Immunology* 49: 578-584.

italics added.[28] Another study concluded, "We think that previous to commercialization of food elaborated with self-insecticide transgenic plants it is necessary to perform toxocological tests to demonstrate the safety of Cry1A proteins for the mucosal tissue and for the immunological system of animals."[29] Such tests have never been carried out on GE Bt-corn.

- **Corn allergen gene turned on as result of genetic engineering.** A carefully designed study involved growing Monsanto's Bt corn varieties, MON 810, in a growth chamber along with its near isoline (corn variety engineered to produce MON 810). Since MON 810 and its near isoline are grown in the same environment, the only difference in the plants will be due to the effect of genetic engineering. This was a proteomic study, which is a study of the expressed proteins, not just of the protein(s) expressed as a result of genetic engineering. Proteomic studies are a good way to detect unintended effects associated with genetic engineering, particularly the disruptive effects due to the random insertion of a transgene. The study found that 43 proteins in the MON 810 plants were significantly disrupted, compared to the non-GE near isoline. As the study notes, "a newly expressed spot (SSP 6711) corresponding to a 50 dDa gamma zein, a well-known corn allergenic protein, has been detected. Moreover, as a major concern, a number of seed storage proteins (such as globulins and vicilin-like embryo storage proteins) exhibited truncated forms having molecular masses significantly lower than the native ones."[30] The safety implications of the truncated seed storage proteins are unknown, as no feeding study was done. So, this study demonstrates that the process of genetic engineering turned on a known corn allergen gene that is normally turned off as well as caused changes to the main proteins found in the seed.

- **Bt corn may cause adverse effects on gut and peripheral immune response.** A carefully designed study (MON 810 and near isoline grown simultaneously in neighboring fields in Landriano, Italy, to control for environmental effects) done by Italian scientists involved feeding a diet containing MON 810 or its near isoline to mice in vulnerable conditions, e.g. weaning and old mice, and looking at a variety of measures of the gut and peripheral immune response. The main finding was that "compared to the control maize, MON810 maize induced alterations in the percentage of T and B cells and of CD4+, CD8+, $\gamma\delta$T, and $\alpha\beta$T subpopulations of weaning and old mice fed for 30 or 90 days, respectively, at the gut and peripheral sites. An increase of serum IL-6, IL-13, IL-12p70, and MIP-1β

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[28] Pg. 147 in Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., Martinez-Gil, A.F., de-la-Riva, G.A., and R. Lopez-Revilla. 2000a. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from *Bacillus thuringiensis* HD 73 in mice. *Brazilian Journal of Medical and Biological Research* 33: 147-155.

[29] Pg. 58 in Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., Martinez-Gil, A.F., de-la-Riva, G.A., and R. Lopez-Revilla. 2000a. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from *Bacillus thuringiensis* HD 73 in mice. *Brazilian Journal of Medical and Biological Research* 33: 147-155.

[30] Pg. 1855 in Zolla, L., Rinalducci, S., Antonioli, P and P.G. Righetti. 2008. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. *Journal of Proteome Research*, 7: 1850-1861. At: http://stopogm.net/webfm_send/288

after MON810 feeding was also found. **These results suggest the importance of the gut and peripheral immune response to GM crop ingestion as well as the age of the consumer in the GMO safety evaluation" bold** added.[31]

- **A meta-analysis of feeding studies involving GE crops suggests health problems and that longer term studies are needed.** A carefully designed meta-analysis was done of 19 published studies involving mammals fed GE corn or soy.[32] The meta-analysis also included the raw data from a number of 90-day-long feeding studies that were obtained as a result of court action or official requests. The data included biochemical blood and urine parameters of mammals eating GE crops with numerous organ weights and histopathology findings. The meta-analysis of all the *in vivo* studies found that the majority of statistically significant results came from parameters involving the liver or kidney. The authors conclude that longer-duration tests are needed, noting that "90-day-long tests are insufficient to evaluate chronic toxicity, and the signs highlighted in the kidneys and livers could be the onset of chronic diseases. However, no minimal length for the tests is yet obligatory for any of the GMOs cultivated on a large scale, and this is socially unacceptable in terms of consumer health protection. We are suggesting that the studies should be improved and prolonged, as well as being made compulsory, and that the sexual hormones should be assessed too, and moreover, reproductive and multigenerational studies ought to be conducted too."[33]

- **A 2005 animal study on transgenic peas found that the genetic engineering process unexpectedly turned a protein that is relatively "safe" into one that causes adverse health effects and increased the potential for adverse effects in other proteins.**[34] A group of Australian scientists looked at the transfer of a gene from beans into peas. The gene codes for a protein, a-amylase inhibitor (aAI), that confers resistance to certain weevil pests. The aAI in raw beans inhibits the action of amylase, an enzyme that degrades starch. So aAI in raw beans can cause gastrointestinal problems in humans. When beans are cooked, the aAI is easily digested and causes no problems. However, when the gene for aAI was inserted into peas, the resultant protein had the same amino acid sequence as the bean aAI, yet the structure of the protein had been subtly altered (through a process called post-translational processing), causing an immunological reaction in mice fed the transgenic peas, but not in mice fed normal beans. The

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[31] Pg. 11533 in Finamore, A., Roselli, M., Britti, S., Monastra, G., Ambra, R., Turrini, A. and E. Mengheri. 2008. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. *Journal of Agriculture and Food Chemistry*, 56: 11533-11539. At: http://www.giovannimonastra.info/documenti_pdf/Monastra_J_Agr_Food_Chem_2.pdf

[32] Séralini, G-E, Mesnage, R., Clair, E., Gress, S., de Vendômois, JS and D. Cellier. Genetically modified crops safety assessments: present limits and possible improvements. *Environmental Sciences Europe*, 23: 10. At: http://www.enveurope.com/content/pdf/2190-4715-23-10.pdf

[33] Pg. 1 in IBID

[34] Prescott, VE, Campbell, PM, Moore, A, Mattes, J, Rothenberg, ME, Foster, PS, Higgins, TJV and SP Hogan. 2005. Transgenic expression of bean α-amylase inhibitor in peas results in altered structure and immunogenicity. *Journal of Agricultural and Food Chemistry*, 53: 9023-9030.

adverse/immunological reaction to the transgenic pea aAI was not mitigated by boiling the peas. The mice fed transgenic peas, in addition to developing an immunological reaction to the pea aAI, also developed an immunological reaction to a number of proteins normally found in peas; mice fed these same proteins from non-engineered peas developed a far smaller immunological response, thus demonstrating that the transgenic pea aAI acts as an adjuvant to increase the immunogenicity of native pea proteins.

This new study involving aAI is extremely important. This study found that moving the same gene between two relatively closely related plants (common beans and peas) can result in a protein that, although it contains the exact same amino acid sequence, is relatively safe in the donor plant (common beans), but is potentially harmful in the recipient plant (peas) and can increase the potential hazardousness of other proteins found in peas. These are all clearly unintended and unexpected effects that clearly result in an adverse health effect.

- **New data confirm unintended and unexpected effect from genetic engineering.** Other studies in the last 5 years have found all sorts of unexpected changes/effects in GE crops. A detailed molecular characterization of various GE crops (three different Bt maizes, an herbicide-tolerant maize, RoundUp Ready soybean, and a male-sterile canola) currently on the market, done in Belgium, has shown that of the transgenic lines looked at, all but one were found to have differences in the molecular characterization in products on the market compared to the original structure reported by the company.[35] Except for the canola, all these reports found that the structure (e.g. molecular characterization) of transgenic inserts as reported by the companies in their initial submission were different than the structure found in subsequent studies. The differences in structure involved rearranged inserts, partial copies of genes inserted, multiple copies of transgenes inserted, scrambling of DNA near the border of the transgenic inserts, etc., suggesting that the transgenic lines are unstable and/or more likely to result in unintended effects. In fact, in virtually all the cases, the SBB/IPH recommends that further analysis "should be done to determine the presence of chimaeric open reading frames in the border integration sequences", e.g. an analysis should be done to see if there are any unexpected proteins being produced.

- **A paper reviewing the food safety issues associated with genetically engineered crops listed a range of documented unintended effects** and concluded that "The development and validation of new profiling methods such

[35] Dr. Moens, with the Service of Biosafety and Biotechnology (SBB) of the Scientific Institute of Public Health (IPH), a government agency reported on the molecular characterization of the genetic map for six transgenic crops: 3 different Bt maizes—Bt 176, Syngenta (www.biosafety.be/TP/MGC_reports/Report_Bt176.pdf); MON 810, Monsanto (www.biosafety.be/TP/MGC_reports/Report_MON810.pdf); Bt11, NorthrupKing (www.biosafety.be/TP/MGC_reports/Report_Bt11.pdf)—a herbicide tolerant maize (LibertyLink maize, Bayer)(www.biosafety.be/TP/MGC_reports/Report_T25.pdf) , glyphosate tolerant soybeans (RoundUp Ready soybeans, Monsanto) (www.biosafety.be/TP/MGC_reports/Report_MON810.pdf) , and a canola engineered for male sterility (Ms8 x Rf3, Bayer Cropscience)

as DNA microarray technology, proteomics, and metabolomics for the identification and characterization of unintended effects, which may occur as a result of the genetic modification, is recommended."[36]

- **An Annex to the Codex Plant Guideline on the assessment of possible allergenicity states that no definitive test exists to accurately predict allergenicity of a given protein:** "At present, there is no definitive test that can be relied upon to predict allergic response in humans to a newly expressed protein."[37] So there is scientific uncertainty around assessment of potential allergenicity of foods derived from GE/GM. Furthermore, a study done by Dutch scientists, using a modified, and more conservative, methodology for screening transgenic proteins for potential allergenicity (e.g. the analysis of sequence homology to known food and environmental allergens) as laid out in the Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (January, 2001), found that a number of transgenic proteins have significant sequence homology to known allergens and recommended further study for a number of these proteins: "Many transgenic proteins have identical stretches of six or seven amino acids in common with allergenic proteins. Most identical stretches are likely to be false positives. As shown in this study, identical stretches can be further screened for relevance by comparison with linear IgE-binding epitopes described in the literature. In the absence of literature values on epitopes, antigenicity prediction by computer aids to select potential antibody binding sites that will need verification of IgE binding by sera tests. **Finally, the positive outcomes of this approach warrant** [papaya ringspot virus coat protein, acetolactate synthase GH50, and glyphosate oxidoreductase] **further clinical testing for potential allergenicity"**[38] **- bold** added. Another study done by Dr. Steven Gendel of the US Food and Drug Administration found that there was significant sequence similarity between a gene in Bt maize and Bt cotton (e.g. Cry1Ab or Cry1Ac) and an egg yolk allergen and recommended further study: "the similarity between Cry1A(b) and vitellogenin might be sufficient to warrant additional evaluation."[39]

While science demonstrates the need to track potential health impacts of genetically engineered food, there is also broad support for labeling genetically engineered food as indicated by the following endorsements by the public health, nursing, medical and healthcare communities:

- In 2001, the American Public Health Association passed a resolution entitled Support of the Labeling of Genetically Modified Foods which "Resolves that APHA declare its

[36] Pg. 503 in Kuiper, HA, Kleter, GA, Notebom, HPJM and EJ Kok. 2001. Assessment of food safety issues related to genetically modified foods. *The Plant Journal*, 27(6): 503-528.
[37] para 2, Annex, CAG/GL 45-2003. At: : http://www.codexalimentarius.net/web/standard_list.do?lang=en
[38] Pg. 1 in: Kleter, GA and ACM Peijnenburg. 2002. Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE – binding linear epitopes of allergens. *BMC Structural Biology*, 2:8. Accessed at http://www.biomedcentral.com/1472-6807/2/8
[39] Pg. 44 in Gendel, S.M. 1998. The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Advances in Food and Nutrition Research*, 42: 44-61.

support that any food product containing genetically modified organisms be so labeled."[40]

- In 2008, the American Nurses Association adopted a resolution on Healthy Food in Health Care, which specifically, "Supports the public's right to know through support of appropriate food labeling including country-of-origin and genetic modification..."[41]

- In 2011, the Illinois Public Health Association adopted a resolution supporting "legislation and/or regulations that require clearly labeling food with genetically engineered ingredients."[42]

- Catholic Healthcare West (a network of 41 hospitals and 10,000 physicians) avoids genetically engineered food and advocates for public policies that include the labeling of genetically engineered food.[43]

Furthermore, twenty state legislatures have introduced bills to require mandatory labeling of GE foods. (IL, AK, CA, NC, IA, MD, NY, OR, RI, WV, VT, TN, HI, CT, MA, MO, NJ, WA, MI, NH).

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The three main monotheistic religions and gm food technology: an overview of perspectives

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Abstract

Background: Public acceptance of genetically modified crops is partly rooted in religious views. However, the views of different religions and their potential influence on consumers' decisions have not been systematically examined and summarized in a brief overview. We review the positions of the Judaism, Islam and Christianity – the three major monotheistic religions to which more than 55% of humanity adheres to – on the controversies aroused by GM technology.

Discussion: The article establishes that there is no overarching consensus within the three religions. Overall, however, it appears that mainstream theology in all three religions increasingly tends towards acceptance of GM technology per se, on performing GM research, and on consumption of GM foods. These more liberal approaches, however, are predicated on there being rigorous scientific, ethical and regulatory scrutiny of research and development of such products, and that these products are properly labeled.

Summary: We conclude that there are several other interests competing with the influence exerted on consumers by religion. These include the media, environmental activists, scientists and the food industry, all of which function as sources of information and shapers of perception for consumers.

Background

In 1999, the Church of England issued a statement that "religious traditions, which are reservoirs of wisdom accumulated and sifted over the centuries, have a vital role to play in helping society to reach the right conclusions" about the genetic modification (GM) of food crops [1]. We know that public acceptance of GM food technology is a crucial issue in the field. Whilst public acceptance is rooted, in part, in religious views, to our knowledge the views of different religions and their potential influence on consumers' decisions have not been systematically examined in a single overview article. In view of the interest and controversy generated by GM food technology, we present a brief overview of relevant positions articulated by religious leaders representing different faith communities and secular commentators – academics, especially scholars who also double as experts on issues relating to these three religious traditions, scientists and adherents of these monotheistic religions – on GM food technology. Specifically, we focus on the world's three major mono-

theistic religions: Judaism, Islam and Christianity, whose adherents, who mostly live in developing countries, collectively constitute more than 55% of the world population.

Discussion

In recent years, the genetic modification of food crops has become a controversial issue in global trade and development [2]. A Genetically Modified Organism (GMO) is one whose genetic structure has undergone a deliberate re-engineering or alteration. For the purpose of this paper, the process involves the introduction of a foreign gene that enables the host organism to manifest specific qualities conferred by the gene [3-5].

Since the introduction of the genetically engineered Calgene's Flavr Savr tomato into the American market in the early 1990s [6], a wide range of new GM food crop products have been developed and marketed worldwide [7]. Not surprisingly, the reception of these new food products has been mixed. Some of the criticisms of GM food technology focus on risks to the environment [8], risks to human beings who consume them [9] the possibility of a few multinational companies dominating global food production [10], and the marginalization of farmers in developing and developed countries [11]. Other complaints include the possibility of dependence of Southern countries on the industrialized North for food [12], the loss of the genetic originality of plants and crops from different parts of the world as a result of gene engineering [13], the distortion and destruction of the cell structure of these organisms [14] and improper labeling [15], especially when GM crops and non GM crops are mixed together [16].

Despite these criticisms, in many parts of the world, numerous scholars, governments and international agencies have been consistent in voicing their support for GM food technology. They argue that scientists alter the genetic structures of plants in order to confer beneficial properties on them. Such benefits include the enhancement of the quality and quantity of crops to increase their micronutrient content [17], the reduction in the maturation time of seedlings [18], the enhancement of plant resistance to pests and disease [19], the improvement of the adaptability of crops to nutrient deficient soil [20] and the production of proteins for human and animal medicine [21] and the conferment of drought resistance.

The idea that we humans should not be "playing God" is widely held among people of many backgrounds. In the context of GM crops, the idea that transgenesis and the crossing of species barriers constitute "playing God" is obviously a subject worthy of serious attention, even if it cannot be upheld with serious analysis. We perhaps need to refer to ethicists for a more objective analysis of this subject. Bernard Rollin, an ethicist, posits that there is nothing intrinsically wrong with scientists crossing the species barrier given that so many of the world's "moral categories" have been adapted or displaced to meet the challenges of our technologically driven contemporary world [22]. Although, many ethicists would not agree with this argument, Rollin's position is consistent with the official position of the UK government, articulated in the Polkinghorne Report, which states that whatever gene (whether human, animal or plant) that is integrated into a host genome is in fact a laboratory fabricated version of the original and its development is not a contravention of ethical, cultural, religious norms or social codes [23]. Polkinghorne was both a scientist and a clergyman and we believe that his views, and the views of the committee he chaired, represent a fair analysis of both the science and ethical and moral dimensions of the subject when the report was written in 1993.

The early controversy seems to have settled where policy is concerned, and a number of safeguards have been proposed to mitigate the risks mentioned above. The European Union (EU), while accepting GM food technology, requires that every food product produced from a genetically modified organism must be labeled accordingly [24]. The position of the World Health Organization (WHO), an organ of the United Nations, is that humankind "could benefit enormously from biotechnology, for example, from an increase in the nutrient content of foods, decreased allergenicity and more efficient food production" [25]. The WHO also argues that any technology involved in food production must be thoroughly evaluated to ensure that concerns about issues such as food, human health and the environment are addressed in a holistic and all-inclusive manner. The same point is emphasized in the Report of the African Union's high-level panel on modern biotechnology, "Freedom to Innovate" [26].

In addition to the positions of international agencies, governmental and non-governmental bodies on GM food technology, religious leaders have attempted to play a role in guiding consumers about GM food technology. For some people religion and the guidance of religious authorities continues to exert a powerful influence on cultural and ethical conventions, especially in developing countries, where research viewed by scientists as being purely scientific and experimental, may be considered inimical and threatening to people's religious convictions and practices.

Perspectives on GM technology in Judaism, Islam and Christianity

Judaism

Within Judaism, the interpretation of life is based on the postulations of different Rabbis, whose moral authority stems from their in-depth understanding of the Divine as contained in the *Torah*, the Hebrew bible, in response to questions of social significance [27]. In a 2005 commentary on GM food technology, Esra Galun, a respected Jewish Professor of Plant Sciences at the Weizmann Institute of Plant Sciences, who is an expert on Jewish religious prescriptions on plants and food crops recognizes that determining whether it is good to develop genetically modified food crops is fraught with problems [28]. Galun refers to two other Jewish philosophers and religious scholars, E. Goldschmidt and A. Maoz, who submit that, based on Jewish religious laws and traditions, the development of transgenic plants by researchers are permissible if they are not directly prohibited by God and if the research will benefit mankind. Another Jewish Rabbi, Akira Wolff [29], supports this view when he states that Jewish tradition believes that man was created in God's image and this affords him the opportunity of partnering with God in the perfection of everything in the world. According to him, Jewish law (*Halacha*) accepts genetic engineering to save and prolong human life as well as increase the quality or quantity of the world's food supply. On the biblical prohibition of *Kilayim*, or mixing of different species of animals and plants, Wolff believes that God does not prohibit the genetic modification of food crops. In concluding his paper, Wolff states "man may manipulate the creation (of God) ... [but] all the legally permitted actions must bring the world closer to perfection and not further away".

In contrast, Michael Green, a British based Jewish commentator, who espouses Orthodox Judaism, argues that there is no consensus within Judaism about GM food technology and he cites a prominent Jewish environmental group in the United States, the Teva Learning Centre (TLC), to support his position. The TLC believes that the GM food technology is a violation of *Kilayim*, the mixed breeding of crops or livestock [30]. Green also refers to two bible verses, Leviticus 19:19 and Deuteronomy 22:9–11, where God prohibits the mixing of species, as proofs that God made "distinctions in the natural world", which Jews must not breech by eating GM food or engaging in GM food research. Green believes that genetic engineering in its entirety endangers nature and human beings. Similarly, in a paper published in 2000, a Conservative Jewish Rabbi, Lawrence Troster, argues that religious traditions should be more cautious before endorsing genetically modified foods. He calls for an acknowledgement of humankind's "limitations in the face of the depth and grandeur of the order of creation" [31].

The different positions on the issue of GM food technology and GM food products and how they affect the average Jew is also discussed in an article entitled "Are Genetically Modified Foods Kosher?" [32], written by Rabbi Tzvi Freeman. Freeman explicitly states that the controversy about whether Jews can eat GM food or engage in GM research stems from the postulations of two renowned Jewish Rabbis, Moshe Ben Nachman and Yehuda Lowe. According to Freeman, Nachman, a medieval Rabbi, argues that God has given humankind the right to dominate and use any of God's creation "but not to disturb its fundamental nature". However, Lowe, who wrote his own interpretations of the *Torah* about three hundred years after Nachman, argues that "any change that human beings introduce into the world already existed in potential when the world was created. All the humans do is bring that potential into activity". Thus, while acknowledging the divergent Jewish positions on the modification of food crops, Freeman emphasizes the need for Jews to look at the health and environmental implications of GM food technology and through such scrutiny seek answers to the question of whether their introduction into the human food supply is actually beneficial or detrimental to the environment and humankind.

The divergence in the views of these Jewish religious leaders, scholars and commentators shows that there is no universal agreement within Judaism on whether Jews can eat GM food products or engage in research in the area of GM food technology.

Islam

Islam is made up of two major branches, Sunni and Shia, distinguished by some doctrinal and historical differences [33]. However, despite these differences, the rulings on modern biological and technological issues tend to be quite similar [34]. At a seminar in Kuwait on genetics and genetic engineering in October 1998, a group of Muslim intellectuals concluded that although there are fears about the possibility of the harmful effects of GM food technology and GM food products on human beings and the environment, there are no laws within Islam which stop the genetic modification of food crops and animals [35]. The Islamic Organization for Medical Sciences in collaboration with the Islamic Fiqh Academy, Jeddah, the World Health Organization's Eastern Mediterranean Regional Office, Alexandria, and the Islamic Education, Science and Cultural Organisation (ISESCO) organized the seminar. Worthy of note is the involvement of the Islamic Fiqh Academy, which is an Academy for advanced study of Islam and which was established by the Organization of Islamic Conference (OIC) in 1988 and which is administered by a body of Islamic clerics. The above conclusion reflects the widely held views of most scientifically informed Muslim scholars, whether Sunni or Shia. Thus it

is noteworthy that scientists in Islamic countries like Egypt and Indonesia (the world's largest Muslim country), are actively manipulating plant genes in a variety of ways. In fact, in 2003, the Indonesian Ulemas Council (MUI) approved the importation and consumption of genetically modified food products by Indonesian Muslims [36].

Ibrahim Syed, an Islamic cleric and the President of the Islamic Research Foundation International, an amalgamation of different Islamic religious groups, is regarded as a leading expert on the interpretation of the Quran in the light of recent advances in the area of modern technology [37]. He has written about the consensus among Muslim scholars that the *Quranic* verse forbidding man from defacing God's creation "cannot be invoked as a total and radical ban on genetic engineering ... If carried too far, it would conflict with many forms of curative surgery that also entail some change in God's creation" [38]. Syed enjoins African and Asian countries, with large Muslim populations, to "reject the propaganda of extremist groups" campaigning against genetic engineering and these new technologies and to embrace them wholeheartedly.

In her own contribution to the discourse, a female Muslim scholar, Fatima Agha al-Hayani, who has written and commented on several aspects of the Islamic religion, contends that Muslims must ensure that genetic modification "may remain mercy-driven" and promote righteousness [39]. She believes GM food technology has the ability "to carry God's work, alleviate hunger and suffering, secure justice and equity for everyone". Therefore, Muslims "must keep up with the new research and discoveries and make connections within the scientific fields". However, the different perspectives on GM food technology within the Muslim world are obvious in a letter written in October 2006 to the British government by Majid Katme, on behalf of the United Kingdom Islamic Medical Association. Katme, a highly respected personality within the Muslim community in the United Kingdom [40] quotes copiously from the *Quran* and asserts that there is no need for genetic modification of food crops because God created everything perfectly and man does not have any right to manipulate anything that God has created using His divine wisdom. He also states that the *Quran* contains several verses, prohibiting man from tampering with God's creation. He ends the letter by emphasizing the position of members of the United Kingdom Islamic Medical Association that there are no benefits that would accrue to Britain from GM food production [41]. Thus, even within Islam, there is no consensus by religious scholars and commentators on whether the *Quran* accepts genetic modification of food crops and the consumption of GM food products by Muslims.

Christianity

The Catholic Church is the largest Christian denomination in the world [42], with all significant matters of theology and Canon Law decided within the Vatican, under the ultimate direction of the Pope [43]. Nevertheless, there is flexibility among various bishops and experts that are well tolerated within the greater Church so long as they do not conflict with fundamental teachings. Thus theological matters of social significance, such as GM crops, may follow different paths such as:

(1) a no "official" Vatican position;

(2) a limited "policy statement or interpretation of scripture or traditions;

(3)or formal theological positions, published in the form of Papal encyclicals developed by the Congregation for the Doctrine of the Faith, a Vatican-based body whose role is to provide formal interpretations in the case of socially relevant issues, such as abortion or euthanasia.

In 2003, the head of the Pontifical Council for Justice and Peace, based at the Vatican, Cardinal Renato Martino, asserted that the Catholic Church supports genetic modification of food crops as an answer for world starvation and malnutrition and because "scientific progress was part of the divine plan" [44,45]. Martino's statement aligns with a papal address by John Paul II in November 2000, in which he states the Vatican's support for the use of biotechnology in agricultural production as long as the "research is submitted beforehand to rigorous scientific and ethical examination". While Benedict XVI, who succeeded John Paul II as Pope, has condemned human genetic engineering, he has not made any categorical statements on GM food technology.

In 2001, the Pontifical Academy of Sciences, (PAS) an influential Catholic organization, published the proceedings of 2 conferences that it organized in 1999 and 2000 on the "Sciences and the future of Mankind". The PAS argues that it is imperative that new or modern technologies be developed to assist in the improvement of agriculture in developing countries as well as help in feeding the world's hungry people who are increasing daily as a result of the rapid expansion of the world's population. The organization is of the opinion that the genetic modification of crops is not a new phenomenon having been in existence for about 10,000 years. However, the organization also advocates for the close cooperation of scientists, governments and farmers to ensure that genetically modified crops are safe for human consumption, especially since modern science has developed novel means for detecting and removing allergens in crops. From the perspective of the PAS, the benefits of genetically modified

crops are immense as they facilitate the actualization of the global goal and desire "to develop plants that can produce larger yields of healthier food under sustainable conditions with an acceptable level of risk" [46]. Recently, scientists at a 2009 conference organized by the PAS came to the conclusion that genetically modified crops "offer food safety and security, better health and environmental sustainability" as a solution to the hunger and poverty ravaging different parts of the world [47].

However, there are certain organizations within the church that are anti GM crops and who espouse positions that are different from the views of Cardinal Renato Martino and Pope John Paul II. These groups believe that the pro-GM lobby has been able to infiltrate the Vatican to enlist its support for the genetic modification of plants. One of such "dissident" groups is the St Columban's Mission Society, which is an Order of Catholic Priests. In recent times, the Columban society has criticized the Pontifical Academy of Science for cooperating with the US embassy to the Vatican to host a pro-GM conference entitled "Feeding the World: The Moral Imperative of Biotechnology". Father Sean McDonagh, an Irish Columban Priest and ecologist has been vociferous in arguing against the support of the Vatican and its Pontifical Academy of the Sciences for GM food technology. According to McDonagh, "All the experts at Catholic development agencies have taken the position that this is not the way to address food security, and that there's no magic bullet for hunger. What's needed is land reform, financial aid to small-scale farmers, markets where they can get value so they're not caught by the middle man. I've spent 40 years at this sort of work, and I know that's the way forward" [48].

The Church of England, which is also known as the Anglican Church, also avers that "human discovery and invention can be thought of as resulting from the exercise of God-given powers of mind and reason". In effect, scientists who are human beings are exercising their qualities as "images of God", who have been divinely endowed to intervene in "natural processes" [49]. The Church of England believes that genetically modified crops must be properly labeled so as to afford "consumers a legitimate degree of informed choice".

It is pertinent to note that there are also differences within the Anglican Church on the issue of GM food technology. While the worldwide head of the church, the Archbishop of Canterbury, is based in England, where he serves as the head of the church in England, there are branches of the Anglican Church in different parts of the world, mostly in countries formerly colonized by Britain. These national branches are very independent and the congregational meetings of the Presiding Archbishops of the different national branches in England, called the Lambeth Council, simply serve as a means of sustaining the links between these different branches of the worldwide Anglican Communion. In fact, the Archbishop of Canterbury is not in a position to impose the views of the English branch of the church on the other members of the Anglican Communion. A good case in point is a statement credited to a former Anglican Archbishop of Cape Town, Njongonkulu Ndungane, who argues against the introduction of GM foods not only in South Africa but throughout Africa. Ndungane is of the view that Africans do not need genetically engineered food. He believes that it is not safe for human consumption and the African farming systems. He asserts that GM food crops would lead to a reduction in jobs, increase African dependence on the countries of the North and destroy biodiversity [50].

In January 2002, the Conference of European Churches (CEC) presented the outcome of the critical examination of the genetically modified food controversy by its Church and Society Commission. The CEC comprises 126 churches, which belong to different Christian traditions (Protestant, Orthodox, Anglican and Old Catholic). The report shows that these Christian churches agree to the introduction of GM food technology on the premise that it is important to establish a "theology of creation" that properly balances research in the area of biotechnology with a genuine concern for everything created by God, which encompasses the whole of humanity and nature in its entirety [51]. The major highlight of the CEC report is its affirmation that the genetic alteration of plants is consistent with biblical teaching. The report further states that although nature belongs to God, it is not sacred and it can be manipulated for the benefit of humankind. What this suggests is that in the opinion of the theologians and scholars who wrote the report, GM food technology is acceptable, as long as scientists remain within specified ethical and moral limits.

Dialectically opposed to the position of the Conference of European Churches is another Christian ecumenical body, the World Council of Churches (WCC), which is based in Geneva. It is a fellowship of churches from more than 120 countries. In June 2005, its Working Group on Genetic Engineering of the Justice, Peace and Creation team published a document entitled "Caring for Life: Genetics, Agriculture and Human Life". The report concluded that it is unethical, from a Christian perspective, for scientists to dabble in the genetic modification of food crops. The position of the working group members is reflected towards the end of the document, where they aver that "GE messes with life, messes with truth, messes with our common inheritance (i.e. human culture and biodiversity), messes with justice, messes with human

health, messes with the lives of peasant farmers in developing countries and the relationship between human beings and other forms of life" [52].

In the concluding segment of the article, Christians scientists who work for companies involved in genetic engineering and who believe in the bible's core message of truth and justice are enjoined to "become whistle-blowers and conscientious objectors" to any research in the field.

Our brief overview of religious perspectives about GM foods suggests that there is no overarching consensus on the permissibility of GM technology, performing of GM research, or consumption of GM foods within the world's three main monotheistic religious traditions. Overall however, it appears that mainstream theology in the world's monotheistic religions accepts the genetic modification of food crops, performing GM research and consuming GM foods as long as there is adequate scientific, ethical and regulatory scrutiny of research and development of such products, and they are properly labeled. The potential implications of such support for the genetic engineering of plants are diverse and range from increasing awareness in humankind's creative ingenuity as well as influencing government policies on issues like food security, international trade and the reduction of poverty.

In today's complex world, in spite of the pervasive presence of religious institutions, the ethos of life is gradually tilting towards individualism and materialism. Djamchid Assadi, a Professor of Marketing and Communication at the American University of Paris, argues that in this age where the manipulation of every aspect of nature by scientists is seen as a triumph and a celebration of humankind's intellectual achievements, religion is less influential in contemporary secular societies than it once was. According to Assadi, unlike the pre-modern period when religion constituted the prevailing ethos around which life revolved, the postmodern era is dominated by "rationalization, meaning the adoption of norms and values emphasizing effectiveness, efficiency and cost benefit equations ..." [53].

Thus, questions about the appropriateness of GM food technology that might once have been legislated upon by religious institutions may ultimately be settled by individual consumers, particularly those who face hunger and uncertain food security, while struggling to survive in a harsh, hostile, volatile and increasingly secular world, where life changing decisions are increasingly no longer being left alone in the esoteric world of the divine and the supernatural [54,55]. This is borne out by the work of Ferdaus Hossain and Benjamin Onyango [56], who contend that the information provided by governments, the media, industry and scientists on biotechnology confuses the consumers. In a survey they conducted to determine consumer acceptance of nutritionally enhanced genetically modified foods, they discover that it is how the individual consumer perceives the risks and benefits of GM crops based on sundry sources of information that actually determines the acceptance or non-acceptance of GM food products. Other studies on consumer acceptance of GM crops [57-60] also echo this view.

In a recent publication, Arthur Einsele [61] has observed a gap between science and perception with regards to GM food products. He concludes that most people have very little understanding of the general facts of what genetic engineering entails and argues that the benefits of GM food technology should be made "literally visible". He posits that people would have to realize the benefits of the genetic modification of food crops before they can accept it. Consumers must also be made to understand, in a "factual, user friendly" manner, that some of the adverse consequences of GM food technology, suggested by its opponents, have not materialized.

Summary

Based on our analysis, we reach these conclusions: First, there is no consensus on whether GM food technology should be banned or accepted by the religious groups discussed. Second, there is also no monolithic view of beliefs within each religion with respect to GM food technology, a situation, which gives room for different interpretations of issues. Third, there is no agreement on what should be prescribed for the followers of each religion with regards to GM food products and the comments by the religious leaders are intended to simply provide guidance about GM food technology. Fourth, competing with the influence exerted on consumers by religion are several other interests like the media, environmental activists, scientists and the food industry, all of which function as sources of information for consumers. Thus, these religions, while assisting adherents in forming opinions, can only be one of the many factors that can be expected to influence consumers' decisions on GM food technology.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EBO, PAS and ASD jointly participated in conceiving the study and developing its content. All authors read and approved the final manuscript.

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Faith & GMOs

Faith and GMOs: Christian, Jewish, and Hindu Congregations Urged To Vote Yes On 37

These Christian, Jewish, and Hindu leaders are urging their congregations to vote for Proposition 37, which would require labeling of genetically engineered foods sold in California. Several clergy appear in videotaped interviews here. There are many reasons why religious leaders support labeling:

1. **The simple acknowledgement that it is man's God-given right to know what we're putting in our bodies and feeding our children.**
2. **Concerns about the health dangers of genetically engineered foods, which have not been adequately studied for their health impacts. (Several studies do show serious health problems.)**
3. **Environmental impacts of genetically modified plants, which contradict man's role as steward of the land.**
4. **The disproportionate risk to low income and inner city residents, who have less access to organic and unprocessed foods.**
5. **Spiritual concerns that since the technology transfers genes between species and creates combinations of organisms that could never naturally occur in nature, it is a violation of God's law.**

Widespread religious enthusiasm for Prop 37 has been expected. Not only do 91% of Americans want GMOs labeled, religious bodies around the world have long supported mandatory labeling, which is already enjoyed by the people of about 50 countries. Some religious bodies go further. The current policy on genetics of the World Council of Churches, for example, calls on people to "Build partnerships with civil society, peoples' movements, farmers and indigenous peoples to oppose genetic engineering in agriculture."

Christian

The United Methodist Church, the Presbyterian Church USA, and a study committee of the **Evangelical Lutheran Church** have all called for genetically engineered foods to be labeled. The **California Council of Churches** is an endorser to Prop 37. And the **World Council of Churches**, an ecumenical body made up of more than 300 denominations from around the world, warned that the failure to label is a special kind of lying. They write: "…the refusal to allow the labeling of GMOs is itself a hiding of the truth, but also makes it impossible to ensure the integrity of the trade in food." In their 2006 statement on Caring For Life, the Council urged its members to fight for labeling for the health and well being of this and future generations.

Rev. Lyndon Harris, the Los Angeles-based Episcopal Priest who was in charge of St. Paul's Chapel across the street from the World Trade Center, points out that "the Global Anglican Communion has come out against Genetically Modified Organisms." **The General Convention of the Episcopal Church** "support(s) the rights of consumers to know the source and content of their food stuffs," and Rev. Harris agrees. "We have a right to know. I am encouraging all involved to work to have GMOs labeled—Proposition 37 in the state of California."

Rev. Harris, who ran a rescue operation after the World Trade Center attack, also has several concerns "about the proliferation about GMOs in our food supply." He says, "If the science, as it indicates, is true, there are serious risks for consuming genetically modified organisms." Rev. Harris avoids buying GMOs and shares his concerns with others, "especially people who are having illness and disease."

He is concerned about the mixing of genes between plants and animals, and about the lack of safety studies conducted on GMOs before they are placed into our diet. "It's one thing to experiment," he says, but "quite another thing to introduce genetically modified organisms GMOs into the food supply without giving due lab testing."

Rev. Dr. Dudley D. Chatman, pastor of the Greater Community Missionary Baptist church in Pacoima, California, doesn't think it's fair to give people food without disclosing what's in it. He says, "I would vote for putting a GMO label on the can, the bottle, on whatever you are eating so you have a choice." From a religious perspective, he says, "We definitely and positively want truth. And to be untrue to me, and not telling me what I'm eating, is definitely a sin." Beyond labeling, Rev. Chatman, like many other Christian leaders, opposes the practice of genetically engineering our food from a religious perspective. "It's abominable," he says. "I like the way God made the stuff in the first place. It's just right. … Everything is so well organized and so well fixed, that hey, why fix what's already working." To his congregation, he says, "if there's any way possible, you should get there to vote to make sure food is labeled when they have GMO ingredients in it. I will vote YES on Prop 37."

Reverend Peter H. Rood of the Holy Nativity Episcopal Church in Westchester, CA, says, "We have to be informed…. I intend to vote yes on Prop 37." He invites those in his religious community to do the same. Rev. Rood is also concerned about the lack of awareness about GMOs in general, and is predisposed against the use of genetically engineered foods as a whole. "It takes my breath away when I think about how many folks in my congregation have no idea." He adds, "It means as a pastor, I'm just going to have to educate all the more to raise consciousness and have folks be active in taking responsibility to stand against this practice."

Jewish

Rabbi Elihu Gevirtz, a biologist and member of Netiaya Council, says that when tomatoes, corn and other fruits and vegetables are produced with genes from pork or shellfish or scorpions, which are not kosher, he needs the foods labeled as GMO in order to avoid them. “If you can’t trust the food that you eat, how can you take care of your children? Labeling food as GMO enables us to make a conscious choice about the content of our food.”

He also says, “The Torah tells us clearly not to put different species together. GMOs are dangerous spiritually. They are a symptom of a spiritual crisis for humanity in which we have overstepped our boundaries. It is not humanity's role to create new species; it is God's.”

Hindu

Swami Ishwarananda, from Chinmaya Mission in Los Angeles, believes that genetic engineering interferes with the natural food that is made by God. As such, “It’s not good for the body.” The Swami says the ancient Vedic practice of Ayurvedic medicine “starts with the right kind of food.” But with genetic engineering, “when certain such modifications in the very structure of the food is done, we have no clue about whether it is the right thing to eat at all or not.”

He considers GMOs to be dangerous to health and advises his congregation, “Please do not consume them. . . . For that,” he says, “labeling is a must. We should support that proposition [37].”

Various religious leaders and experts also acknowledge that in spite of the biotech industry claims to the contrary, GMOs are not needed to feed the world, do not increase average yields, do not reduce the use of agricultural chemicals, and have not been adequately tested or proven safe. Furthermore, Prop 37 will not increase costs to consumers, result in excessive lawsuits, or hurt farmer incomes.

Want to know more about the health and environmental risks of GMOs?

To learn more about GMOs please watch the seminal documentary, Genetic Roulette, directed by internationally recognized authority on GMOs, Jeffrey Smith. With on-camera testimonials from scientists, doctors and pediatricians discussing gmo consumption, this film is free for only a short time and promises to be a transformative experience.

You can view a 10-minute teaser here:

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“After I watched this [Genetic Roulette, The Movie], I opened my refrigerator and said to myself, what have I been eating?”—Reverend Daniel Buford, Allen-Temple, Oakland, CA

Institute Responsible Technology (IRT) is offering free screenings at your venue upon request. IRT can also assist in getting a credited speaker on the topic of GMOs to talk to your congregations and in your communities. Let’s keep the discussion going!

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Christian Faith Leaders, GMOs, and Prop 37/Food Labeling

Why Christian Faith Leaders are urging passage of Prop 37 and the labeling of genetically modified food(GMO)

The World Council Of Churches, The United Methodist Church, The Presbyterian Church USA, and a study committee of the Evangelical Lutheran Church have all called for genetically engineered organisms to be labeled. In addition, some bodies, such as the California Council of Churches and National Office Of Presbyterian Church **have specifically endorsed Prop 37.**

Various religious leaders and experts also acknowledge that in spite of the biotech industry claims to the contrary, GMOs are not needed to feed the world, do not increase average yields, do not reduce the use of agricultural chemicals, and have not been adequately tested or proven safe. These tenants served as the basis of the biotech industry's promises to the Christian Community regarding the value of GMOs and have been proven, point-by-point, to fail on all accounts.

Furthermore, while those opposing Prop 37 insist the proposition will increase costs to consumers and result in excessive lawsuits or hurt farmer incomes, again all claims are invalid. It is believed that at best, refusal to label is, as the World Council of Churches describes, "a special kind of lying."

Please consider these crucial statements regarding GMOs and food labeling issued by representatives throughout the Christian community and central to Christian concerns:

It is our God given right to know what is in our food.

The World Council of Churches believes "it is immoral that consumers be left in the dark about what is in their food" And the United Methodist General Conference urges, "that genetically modified crops and genetically engineered or cloned animal products be labeled so that consumers have a choice in which kind of agricultural products they buy."

Jaydee Hanson, Secretary Of United Methodist Caretakers Of God's Creation and prior member of The World Council Of Churches Genetics Committee, is passionate about labeling GMO food and emphasizes, "The number one theological driver in Genesis 2 is that we're called to keep and tend God's creation. That doesn't mean advocating pesticide-promoting plants that are genetically engineered."

Reverend Dr. Dudley D. Chatman, Pastor at the Greater Community Missionary Baptist Church, adds, "I would vote for putting GMO labels on the can, bottle, whatever you're eating or drinking. We send food all over the whole word and people don't know it's modified. I don't think that's fair, me giving you food that you don't know what's in it."

It is important to the stewardship of our land that we treat it with respect and reverence.

The World Council of Churches is committed to building "partnerships with civil society, peoples' movements, farmers and indigenous peoples to oppose genetic engineering in agriculture." The Council is concerned about "the risks of genetic technology that can hardly be calculated when breeding animals and plants." The Council continues, "The negative ecological and social impact on agriculture make the use of this technology doubtful." The Council recognizes that, "Access to food stands on the interface between ecology and economy."

The Episcopal Church strongly advises, "that the farming and processing practices used are healthy and sustainable for all of creation". Further more, The Episcopal Church is "committed to making this a reality." The United Methodist Church cautions, "The negative impacts on food and the environment must be strenuously avoided."

Reverend Peter Rood of the Episcopal Church asserts, "We talk about not stealing but anytime we don't practice sustainability we are stealing from our children, from our children's children and from the earth. There is a benefit that comes from caring for the earth and a learning that comes from gardening, you learn of the vitality of the seed and you

Concerns Regarding Genetically Modified Food

This article in the URL below is a part of the series by the Minnesota Episcopal Environmental Stewardship Commission written in support of our resolution on the spirituality of food, especially its production, which was passed at the 2001 Diocesan Convention and has been submitted to General Convention.

Episcopal Diocese of Minnesota

Christian Faith-Based "Talking Points"

Click on the links below to read what Christian Faith Leaders are saying about GMOs, Prop 37, and Food Labeling:

- World Council of Churches Asks Churches to Oppose Genetic Engineering of Food
- Episcopal Church 2003 Resolutions
- Comments of the Ecumenical Eco-justice Network on Labeling of Genetically Engineering Salmon
- "New Developments in Genetic Science" Resolution section on Agriculture as amended by 2008 United Methodist General Conference.

Talking Points Download

Faith-based outreach.pdf

must stand up for the democracy that people who grow their own food enjoy."

We must protect our health; this is our God-given right and moral responsibility

The World Church Council advises, "Christians involved in medical research to investigate the human health impacts of genetically engineered food."

Reverend Lyndon Harris, founder of the Episcopal Church, Gardens of Forgiveness, agrees, "We have a right to know what we're buying and if the science, as it indicates, is true there are serious health risks for consuming GMOs."

Reverend Lyndon and the World Church Council are not alone. The Global Anglican Communion has come out against genetically modified organisms (GMO). In fact, the Archbishop of Canterbury, Rowen Williams, who is the spiritual leader of the Anglican Communion of which the Episcopal Church is a part, has issued his own concerns and statements about the dangers of GMOs. Further defining their position on genetically modified organisms, the Church of England property in the UK considers it illegal to plant GMOs.

*You can read these sources in their entirety at:

Genetically modified organisms in our food violate God's law.

While The World Council of Churches approves of modern methods of breeding, they stress those methods must "respect the existence of the natural borders of species." The Council feels so strongly about GMO technology and food that it "challenges Christians in the genetic engineering industry to reflect on their work in light of the Gospel and to consider becoming whistle blowers and conscientious objectors." The Council also asks all to "envision the Lord's Supper as a sacrament of resistance against those who would control food."

The United Methodist Church states, "The responsibility of humankind to God's creation challenges us to examine the possibilities of genetic research and technology in a conscientious, careful and responsible way."

Greg Ciola writes in NewsWithViews, "The author of all life is God and according to the first chapter of the Book of Genesis, He created every species to reproduce after its own kind. Thus, there is no room in God's creation for man to step in and start modifying DNA by cross transplanting the genes from one organism or species into the DNA of another. In essence, man is now creating species variants that God never intended to exist. Such alteration of species specificity is a serious violation of God's natural order" He quotes, "*And God said, Let the earth bring forth grass, the herb yielding seed, and the fruit tree yielding fruit after his kind, whose seed is in itself, upon earth: and it was so. And the earth brought forth grass, and herb yielding seed after his kind, and the tree yielding fruit, whose seed was in itself, after his kind: and God saw that it was good*." (Genesis 1:11-12)

This scripture supports Reverend Dr. Dudley D. Chatman, Pastor, Greater Community Missionary Baptist Church, when he states, "I like the way God made the stuff in the first place. It's just right."

Want to know more about the health and environmental risks of GMOs?

To learn more about GMOs please watch the seminal documentary, Genetic Roulette, directed by internationally recognized authority on GMOs, Jeffrey Smith. With on-camera testimonials from scientists, doctors and pediatricians discussing gmo consumption, this film is free for only a short time and promises to be a transformative experience.

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Sources related to the "Christian Faith Leaders, GMOs, and Prop 37/Food Labeling":

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Rev. Lyndon Harris

Rev. Dr. Dudley D. Chatman

Reverend Peter H. Rood

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Ethical Eating: Food & Environmental Justice

2011 Statement of Conscience

Aware of our interdependence, we acknowledge that eating ethically requires us to be mindful of the miracle of life we share with all beings. With gratitude for the food we have received, we strive to choose foods that minimize harm and are protective of the environment, consumers, farmers, and all those involved in food production and distribution.

Environmental justice includes the equitable distribution of both environmental burdens and benefits for populations of residents and workers. Marginalized people have often been able to find housing or work only in areas exposed to environmental pollutants, with consequent negative health and quality of life effects.

As Unitarian Universalists, we are called to address our relationship with food. Our Principles call for recognition of and respect for the other. As we search freely and responsibly for truth, meaning, and spiritual wholeness, we will make a variety of individual choices about food. Ethical eating is the application of our Principles to our food choices. What and how we eat has broad implications for our planet and society. Our values, Principles, and integrity call us to seek compassion, health, and sustainability in the production of food we raise or purchase.

Food production involving growing, processing, packaging, transporting, and distributing food has become a vast worldwide industry. The mass production of food often maximizes production while minimizing price. This mass production has greatly increased food supply, but has resulted in the overuse of fertilizers and pesticides with crops and the mistreatment of animals and workers in food production. Both this overuse and the large waste streams from concentrated animal feeding operations (CAFOs) result in pollution of water, land, and air.

Access to an adequate supply of healthy food and clean water is a basic human need and right. Many people do not have adequate food, while others have a surplus. In many locations, poor distribution of food is a major contributor to hunger and malnutrition. The effects of climate change, weather conditions, and armed conflicts can also expose many people to starvation. Paradoxically, an abundance of food does not guarantee access to healthy food.

We acknowledge that aggressive action needs to be taken that will ensure an adequate food supply for the world population; reduce the use of energy, water, fertilizer, pesticides, and hormones in food production; mitigate climate change; and end the inhumane treatment of animals. These steps call for an evolution of our eating habits to include more locally grown, minimally processed whole foods. We acknowledge that this evolution must respect diversity in cultures, nutritional requirements, and religious practices.

Minimally processed plant-based diets are healthier diets. Some of us believe that it is ethical only to eat plants while others of us believe that it is ethical to eat both plants and animals. We do not call here for a single dietary approach. We encourage a knowledgeable choice of food based on understanding the demands of feeding a growing world population, the health effects of particular foods, and the consequences of production, worker treatment, and transportation methods. We commit to applying this knowledge to both personal and public actions, recognizing that many of us might embark on a dramatic change in eating choices and some might pay more for food that is ethically produced. For congregations, helping congregants gain this understanding and supporting their choices will require a long-term collective process of engagement, education, discernment, and advocacy. Unitarian Universalists aspire to radical hospitality and developing the beloved community. Therefore, we affirm that the natural world exists not for the sole benefit of one nation, one race, one gender, one religion, or even one species, but for all. Working in the defense of mutual interests, Unitarian Universalists acknowledge and accept the challenge of enlarging our circle of moral concern to include all living creatures.

As individuals and as congregations, we recognize the need to examine the impact of our food choices and our practices and make changes that will lighten the burden we place on the world. We also recognize that many food decisions will require us to make trade-offs between competing priorities. These priorities include: taste, selection, price, human health, environmental protection, sustainability, adequate food supply, humane treatment of animals used for food, and fair treatment of farm and food workers.

Environmental concerns include the use of fertilizers, herbicides, pesticides, and hormones and high volumes of animal wastes produced by CAFOs, all of which can contaminate soil, air, and water. Contributors to global warming include the overreliance on fossil fuels for food production; the methane produced by animals, including but not limited to cattle, sheep, and pigs; and the long-distance transport of food. Expanding agriculture and animal farming often removes natural habitats and reduces natural biodiversity. An additional environmental concern is the deterioration of the oceans and their life forms due to overfishing and pollution.

Human Health concerns include producers' use of growth promoters, pesticides, and antibiotics that can affect child development, antibiotic resistance, and other health conditions. Advertising and marketing can encourage overeating, poor food choices, a focus on body image that can contribute to eating disorders, and the use of infant formula in preference to breast feeding.

Concerns about the **Humane Treatment of Animals** include intensive confinement and abuse in CAFOs, and inhumane conditions during production, transport, and slaughter.

Concerns about the **Fair Treatment of Food and Farm Workers** include low pay, poor and unsafe working conditions, exploitation of undocumented workers, and enslavement of others.

Policy concerns include agricultural subsidies that reward the production of certain crops and animal products that are less healthful and environmentally friendly than unsubsidized ones and that penalize small to moderate-sized farming operations. Agricultural subsidies of exported crops have driven small farmers in developing countries off their land. The consequences of agricultural subsidies and mono-cropping include increased gender disparity where women have been the traditional agricultural producers. We recognize replicating corporate agricultural modes in our aid to developing countries is not in the best interest of humanity. We support the development of farming models that safeguard the environment, produce safe foods, provide economic benefits to all economic levels, and create environmentally and economically sustainable models.

Classism, racism, sexism, and other forms of oppression are deeply connected to economic justice, which is a prime determinant of access to food. Some of us will not be able to pay more for ethical food. Others of us will. Yet all of us can have a role in improving the ethics of food. We affirm that the fight for environmental and economic justice is inherently a fight against all forms of oppression. As a result, ethical eating requires different ways of thinking about these issues that reflect their interconnected nature, and we understand that this work will require creativity, patience, and resolve.

Calls To Action

Individual Actions

Recognizing that individual circumstances vary, we aspire to buy, raise, and consume food for ourselves and our families that:

- increases our proportionate consumption of plant-based foods, which increases the global access to calories, provides health benefits, and prevents injuring animals;
- minimizes the pain and suffering of animals by purchasing meat or seafood produced under humane conditions, for those who choose to eat meat or seafood;
- minimizes the negative environmental effects of raising animals or plants by purchasing organically produced food, and seafood certified as responsibly farmed or harvested;
- minimizes transportation-related carbon dioxide emissions by obtaining foods locally produced through home or community gardens, farmers markets, or community supported agriculture (CSA);
- provides farm workers with living wages and safe working environments;
- contributes to social harmony by encouraging communal eating;
- promotes health, consuming food in quantities that do not lead to obesity; and

We advocate for the benefit of animals, plants, food workers, the environment and humanity by:

- purchasing fair trade--certified products as available.
- asking food sellers and producers to label where their products come from to determine distance of transport and whether the products were irradiated or contain Genetically Modified Organisms (GMOs);
- pressing food sellers to require that their suppliers certify the humane treatment of animals;
- supporting legislation that requires the labeling of products that are irradiated or contain Genetically Modified Organisms (GMOs), distribution of adequate ethical food supplies, effective safety inspection of food production, and realignment of agricultural subsidies to support growing more produce and the viability of small farmers; and
- protecting and encouraging organic food production and its producers.

Congregational Actions

As congregations, we aspire to:

- provide and sell more plant-based, organic, locally produced, and fair trade foods at congregational events;
- promote economic accessibility to safe, ethically produced food by organizing members to work for food justice through activities such as: urging grocery chains to locate stores in low income neighborhoods, supporting local food co-ops, helping people obtain food stamps, advocating for increased funding to alleviate hunger, and assisting local meals on wheels and food bank programs;
- support the Unitarian Universalist Service Committee, Unitarian Universalist United Nations Office, and other relevant UU organizations in their efforts to ensure that everyone has adequate nutritious food, produced sustainably;
- provide educational programs for all ages that address the issues of environmental justice, world hunger, gardening, food preparation, and nutrition;
- become Green Sanctuary—accredited and include ethical eating in programs;
- advocate for healthful food for school and other institutional meals; and
- engage in direct action in solidarity with workers and labor advocacy groups to support agricultural and food workers.

With gratitude and reverence for all life, we savor food mindful of all that has contributed to it. We commit ourselves to a more equitable sharing of the earth's bounty.

Last updated on Wednesday, August 24, 2011.

Gun Control

Strong Support for Labeling Modified Foods

By ALLISON KOPICKI
Published: July 27, 2013

Americans overwhelmingly support labeling foods that have been genetically modified or engineered, according to a New York Times poll conducted this year, with 93 percent of respondents saying that foods containing such ingredients should be identified.

Three-quarters of Americans expressed concern about genetically modified organisms in their food, with most of them worried about the effects on people's health.

Thirty-seven percent of those worried about G.M.O.'s said they feared that such foods cause cancer or allergies, although scientific studies continue to show that there is no added risk.

Among those with concerns, 26 percent said these foods are not safe to eat, or are toxic, while 13 percent were worried about environmental problems that they fear might be caused by genetic engineering.

Nearly half of Americans said they were aware that a large amount of the processed or packaged foods they now buy at the grocery store contains genetically modified ingredients. And although just a handful of G.M.O. crops are on the market, about 4 in 10 respondents said they thought that most or a lot of their fruits and vegetables were genetically modified.

Overall concern was higher among women than men, perhaps not surprisingly, as more women identify themselves as the principal grocery shopper in the household.

Americans were almost equally divided about eating genetically modified vegetables,

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fruits and grains, with about half saying they would not eat them.

They were even less comfortable about eating meat from genetically engineered animals: three-quarters said they would not eat G.M.O. fish, and about two-thirds said they would not eat meat that had been modified.

The national telephone poll was conducted from Jan. 24 to 27 with 1,052 adults and has a margin of sampling error of plus or minus three percentage points.


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We constantly interrupt our experiences to make

WORLD »

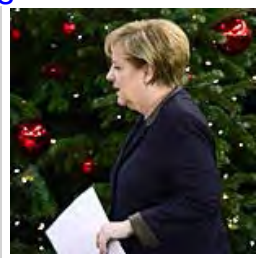
Booksellers Wary About Holiday Sales

Bad Girl Meets Bad Santa

future — that failure will become obsolete.

A Grand Weekend Out for Pennsylvanians

a record of them.



Critics to Merkel: Be Daring

NATIONAL SURVEY OF HEALTHCARE CONSUMERS: GENETICALLY ENGINEERED FOOD

OCTOBER 2010

THOMSON REUTERS™

SURVEY OVERVIEW

Each year, the *Thomson Reuters PULSE™ Healthcare Survey* collects information about health-related behavior and attitudes from more than 100,000 U.S. households. This nationally representative telephone survey is conducted in 11 waves (each a standalone market research study) conducted sequentially throughout the year. Many healthcare topics are covered, including use of medical services, health status, insurance coverage, lifestyle, and current issues. The survey is self-funded and the data it generates are used in Thomson Reuters information products for healthcare professionals, particularly strategic planning and marketing managers in hospitals.

The results depicted below represent responses from 3,025 survey participants interviewed from October 1-13, 2010. The survey questions, which address consumer attitudes toward genetically engineered food, were developed in conjunction with National Public Radio. The margin of error is 1.8 percent.

EXECUTIVE SUMMARY

The survey asked respondents about their awareness of and attitudes towards genetically engineered food. Among those polled, only 25% said they completely understood genetic engineering. More than two-thirds of respondents (69%) said they were aware that genetically engineered foods were currently being sold in stores, but 64% said they are unsure if it is safe.

- 93% of respondents said genetically engineered foods should be labeled.
- 60% said they are willing to eat genetically modified vegetables, fruits, and grains. That number dips to 38% for meat and 35% for fish.
- Awareness of genetically engineered foods increased as income and education levels increased. Only 51% of respondents who earn less than $25,000 said they were aware of genetically engineered foods — compared with 84% of those who earn over $100,000.
- Older respondents are the most willing to eat genetically engineered food. Only 32% of respondents ages 35-64 said they would eat altered fish, compared to 43% of those 65 and over.

SURVEY DATA

Responses in RED are statistically significant.

QUESTION 1: On a scale of 1 to 5 — where 1 is "Do Not Understand at All" and 5 is "Understand Completely" — how well do you understand genetically engineered food?

	1: Not At All	2	3	4	5: Completely
Age					
<35	18.7%	13.6%	26.4%	20.4%	20.9%
35 - 64	14.0%	10.8%	24.0%	23.6%	27.6%
65+	20.7%	10.4%	26.7%	19.6%	22.7%
Total	**16.3%**	**11.5%**	**25.1%**	**22.1%**	**25.0%**
Income					
< $25k	24.9%	13.2%	24.1%	15.0%	22.8%
$25k - $49.9k	17.4%	14.6%	23.9%	23.1%	21.0%
$50k - $99.9k	13.5%	9.7%	25.2%	26.0%	25.6%
$100k+	7.2%	8.7%	28.1%	24.9%	31.1%
Total	**16.3%**	**11.5%**	**25.1%**	**22.1%**	**25.0%**
Education					
High School or Less	30.2%	13.3%	21.9%	14.0%	20.6%
Some College	16.5%	15.3%	28.0%	18.3%	21.8%
College+	11.5%	8.5%	24.2%	27.5%	28.3%
Total	**16.3%**	**11.5%**	**25.1%**	**22.1%**	**25.0%**

QUESTION 2: What is your opinion regarding the safety of genetically engineered foods? Would you say:

1 Genetically engineered foods are not safe?
2 You are unsure of the safety of genetically engineered foods?
3 Genetically engineered foods are safe?

	Not Safe	Unsure	Safe
Age			
<35	12.2%	71.9%	15.9%
35 - 64	16.4%	61.9%	21.7%
65+	12.0%	58.3%	29.7%
Total	**14.6%**	**64.1%**	**21.4%**
Income			
< $25k	10.7%	71.1%	18.2%
$25k - $49.9k	17.3%	64.9%	17.8%
$50k - $99.9k	16.0%	61.4%	22.6%
$100k+	14.8%	57.2%	28.0%
Total	**14.6%**	**64.1%**	**21.4%**
Education			
High School or Less	13.6%	68.8%	17.6%
Some College	13.5%	71.2%	15.3%
College+	15.3%	58.2%	26.5%
Total	**14.6%**	**64.1%**	**21.4%**

QUESTION 3: Do you believe that foods should be labeled to indicate that they have been genetically engineered or contain ingredients that have been genetically engineered? (These results represent the percentage of people who answered yes.)

Age	
<35	94.1%
35 - 64	93.6%
65+	89.7%
Total	**93.1%**
Income	
< $25k	92.5%
$25k - $49.9k	96.1%
$50k - $99.9k	91.5%
$100k+	92.0%
Total	**93.1%**
Education	
High School or Less	95.1%
Some College	95.2%
College+	91.1%
Total	**93.1%**

QUESTION 4: Would you eat the following foods knowing that they have been genetically engineered?

1 Fish
2 Meat
3 Vegetables, fruits, or grains

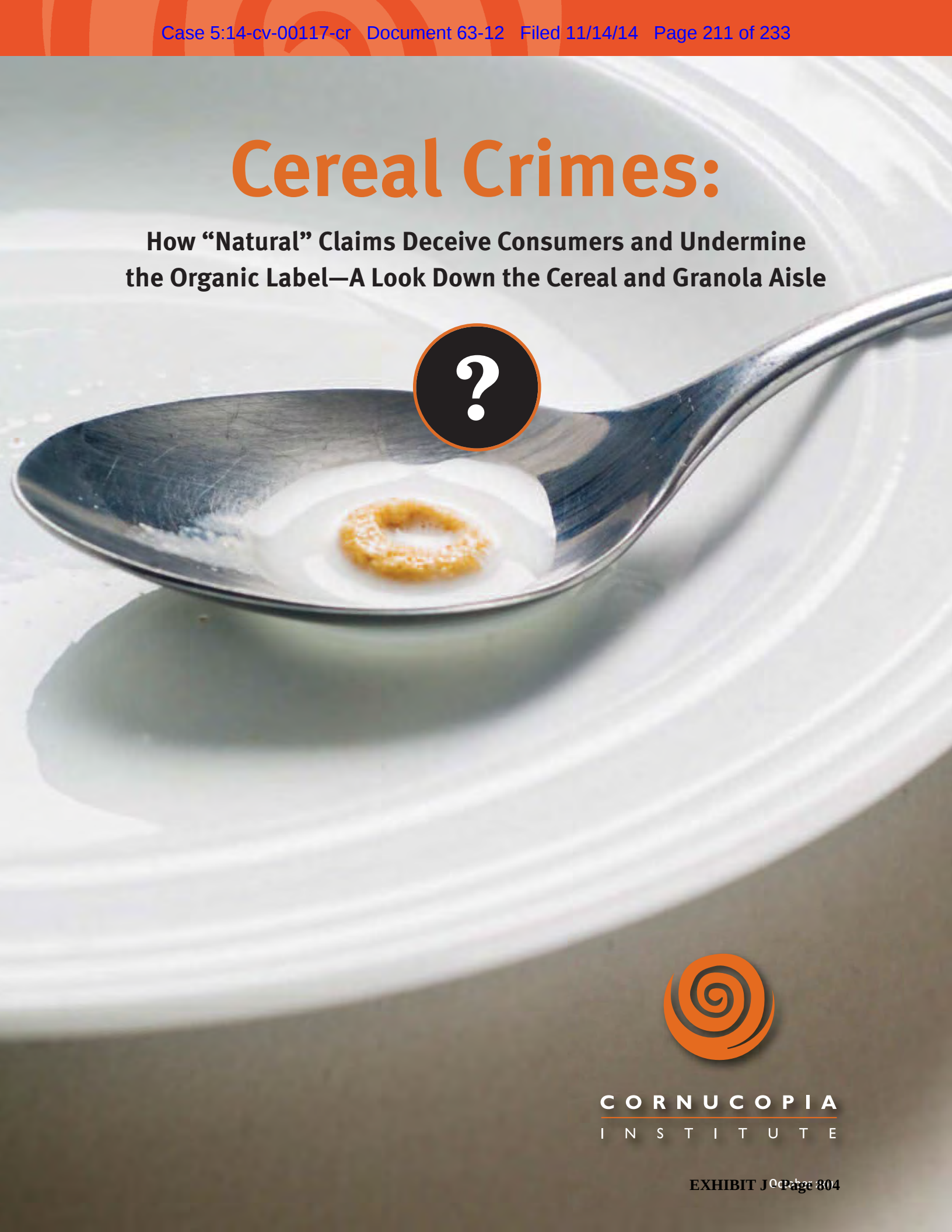
	Fish	Meat	Vegetables, Fruits or Grains
Age			
<35	36.2%	40.6%	65.8%
35 - 64	32.3%	35.7%	56.1%
65+	42.6%	43.2%	63.3%
Total	**35.1%**	**38.3%**	**59.9%**
Income			
< $25k	38.1%	44.2%	59.8%
$25k - $49.9k	30.5%	31.6%	53.8%
$50k - $99.9k	35.2%	39.6%	62.2%
$100k+	37.8%	39.0%	67.6%
Total	**35.1%**	**38.3%**	**59.9%**
Education			
High School or Less	35.3%	38.5%	56.3%
Some College	29.2%	36.3%	53.9%
College+	39.0%	39.8%	65.3%
Total	**35.1%**	**38.3%**	**59.9%**

QUESTION 5: Prior to today, did you know that some of the foods available in stores today have been genetically engineered? (These results represent the percentage of people who answered yes.)

Age	
<35	60.0%
35 - 64	73.9%
65+	68.3%
Total	**69.2%**
Income	
< $25k	51.3%
$25k - $49.9k	68.3%
$50k - $99.9k	77.4%
$100k+	84.3%
Total	**69.2%**
Education	
High School or Less	44.7%
Some College	63.3%
College+	81.4%
Total	**69.2%**

Cereal Crimes:

How "Natural" Claims Deceive Consumers and Undermine the Organic Label—A Look Down the Cereal and Granola Aisle



CORNUCOPIA INSTITUTE

What do the organic standards say about genetically engineered organisms?

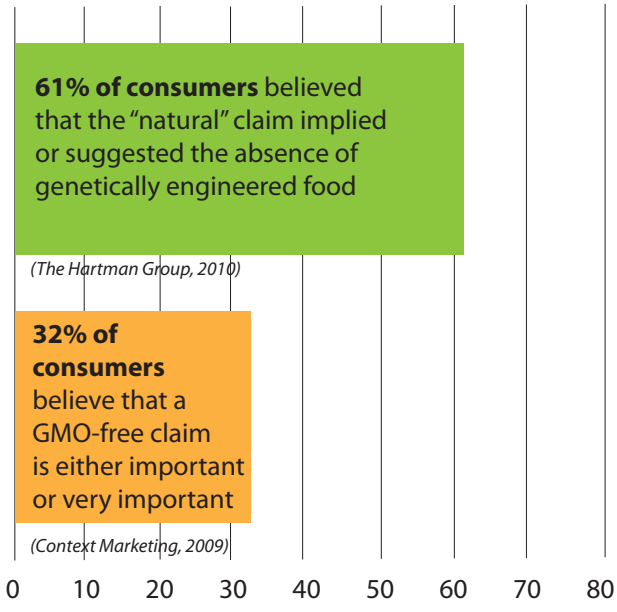
Federal regulations (7 CFR 205.105) define genetic engineering as an "excluded method" for organic production.[31] In other words, the use of genetically engineered seed is strictly prohibited in organic food production, and organic producers must have verifiable practices in place to avoid contact with genetically engineered organisms.

No such prohibition against genetically engineered organisms exists in "natural" standards, especially since every company determines its own definition for "natural" foods.

Consumer expectation regarding the use of GE ingredients in "natural" foods

Research shows that a majority of consumers expect "natural" foods to be free of genetically engineered ingredients, and many also consider the absence of genetically modified organisms (GMOs) to be important.

The 2010 Hartman Group poll found that 61% of consumers erroneously believed that the "natural" claim implied or suggested the absence of genetically engineered foods.[32] According to the 2010 Context Marketing poll, 32% of consumers believe that a GMO-free claim is either important or very important.[33]



Cornucopia tests "natural" cereal for GMOs

To determine whether various brands of non-organic "natural" breakfast cereal are made with genetically engineered ingredients, The Cornucopia Institute sent samples of breakfast cereal to an accredited and highly reputable GMO testing laboratory. Samples were tested for the exact percentage of genetically engineered corn or soybeans, using the most sophisticated and accurate tests commercially available.

The results were stunning. Several breakfast cereal manufacturers that market their foods as "natural," even some that claim to avoid genetically engineered ingredients and are enrolled in the Non-GMO Project, contained high levels of genetically engineered ingredients.

GMO test results

Numerous "natural" products were indeed contaminated with high levels of GE ingredients, sometimes as high as 100%: **Kashi® GoLean®, Mother's® Bumpers®, Nutritious Living® Hi-Lo®, and General Mills Kix®**.[34]

For non-organic "natural" products making "non-GMO" claims, results showed that these claims cannot always be trusted. While **Peace Cereal®** and **Annie's Homegrown®** were indeed free of significant levels of GE ingredients,[35] **Barbara's Bakery® Puffins®** and **Whole Foods' 365® Corn Flakes**, which are both enrolled in the Non-GMO Project contained more than 50% GE corn.

On the other hand, as a control, The Cornucopia Institute also tested Nature's Path® certified organic corn flakes, which were free of significant GE contamination (>0.5%).

These test results underscore the importance of the organic label, which assures consumers that the manufacturer uses only non-genetically engineered ingredients. More extensive testing is necessary to draw conclusions regarding the truthfulness of "non-GMO" claims, but these preliminary results point to several problems. First, manufacturers can claim that they avoid purchasing genetically engineered ingredients, but these claims may be meaningless unless they are verified by a third party, such as an organic certifying agent.

ORGANIC & NATURAL 2012

hartman GROUP

RESEARCH CONSULTING : CUSTOMIZED

Introduction: Years of Knowledge

The *Organic and Natural 2012* report is the latest in our syndicated research that stretches back to 1997.

Times have changed and today we'll discuss some of the highlights from this year's report including:

Shifting meanings of organic and natural to consumers

Motivations and **barriers** to organic use

How consumers **prioritize** organic purchases

The role of **retailers** in the product selection process

Larger **values** Organic symbolizes to today's consumers

METHODOLODY

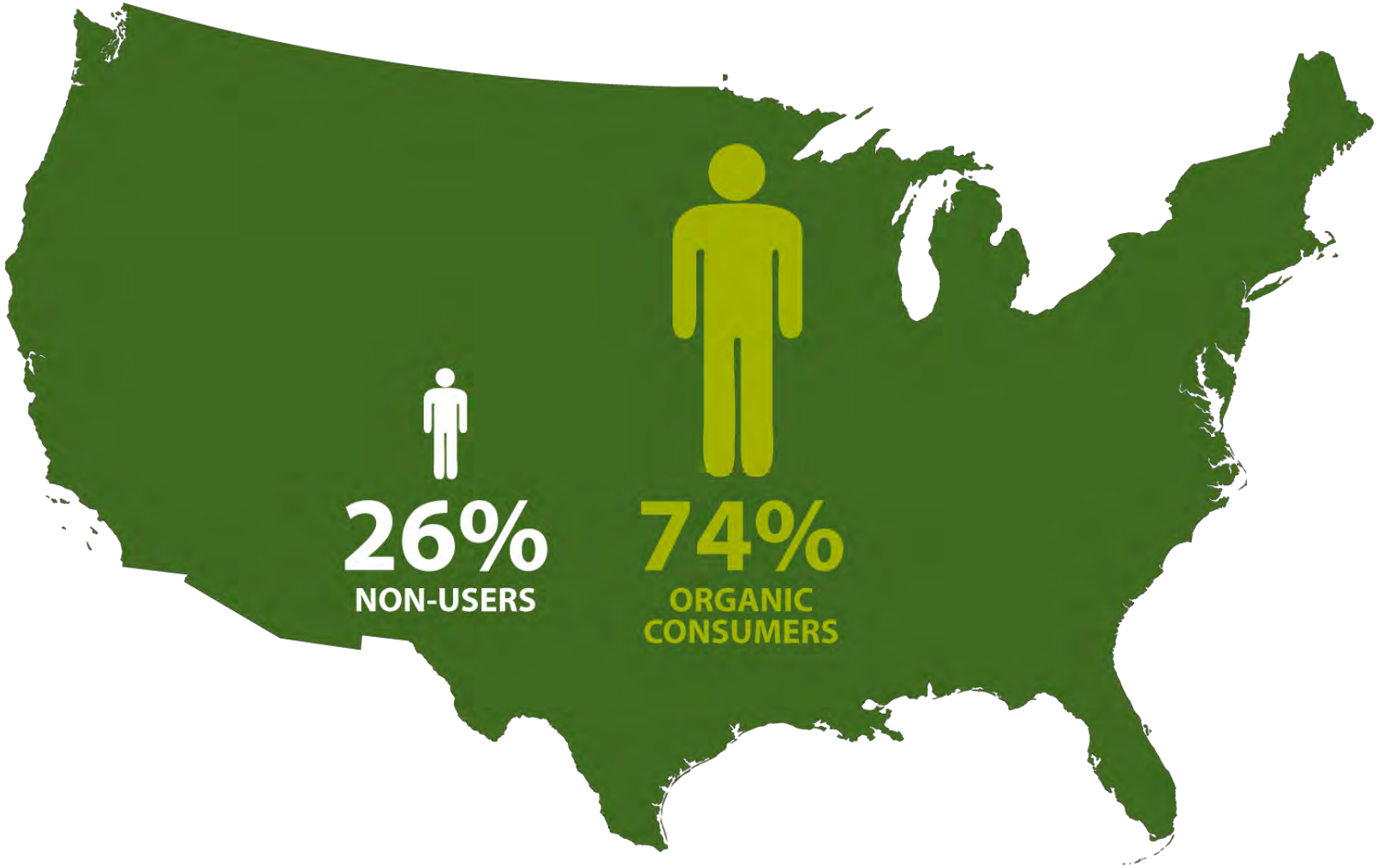
We employed a mix of Qualitative and Quantitative methods

In-depth interviews and ethnographic research groups with a total of 25 consumers. Interviews were held in Seattle and Atlanta, included consumers with various levels of engagement with natural & organic.

An **online survey** of **1,569** U.S. adult (ages 18-69) primary shoppers provided data on such topics.

Fielded July 2012 to nationally representative sampling frame (18-69 y.o.), with sampling error less than ±2.5% at 95% confidence level

Today, about three quarters of U.S. consumers purchase Organic

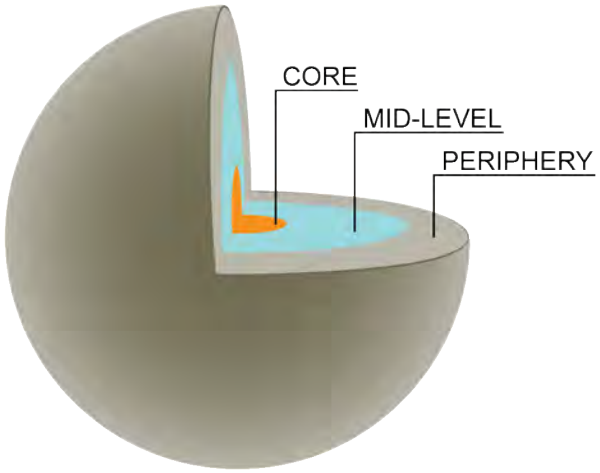


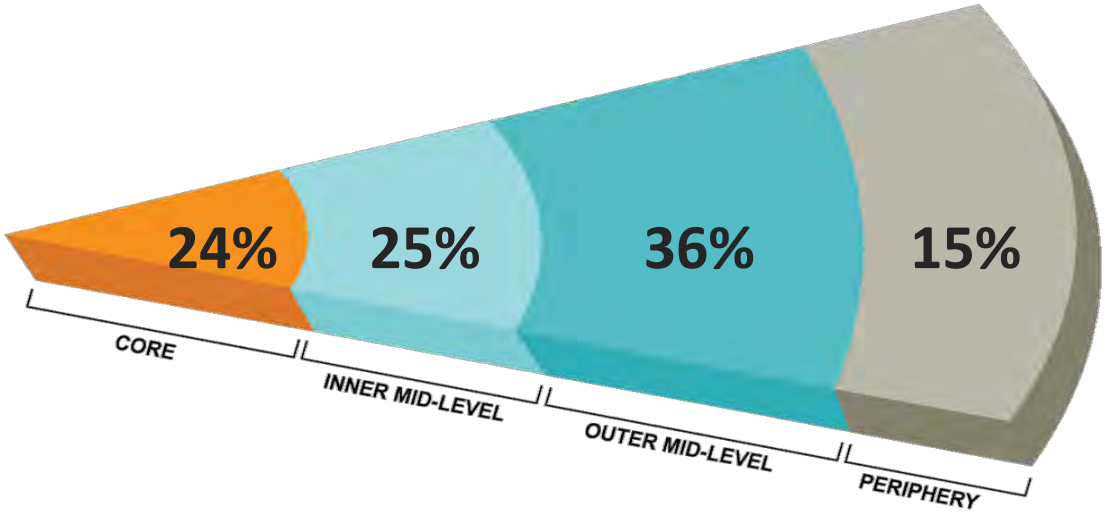
However, consumers purchase organics differently depending on their orientation toward organic

Core is most intensely involved in organics – they are e*arly adopters* and *committed gurus*

Mid-level is the majority of consumers - they are *knowledge seeker* and *experimenters*

Periphery is are the least involved consumers – they are the *dabblers*





MEANINGS OF ORGANIC & NATURAL

As Organics solidify in the mainstream, its meaning has shifted

The rise in organic products has mixed results for consumers:

They continue to associate Organic with the **absence of negatives** in the growing process (64% say it means no pesticides), however…

There is **growing uncertainty** whether mainstream companies can do organic correctly.

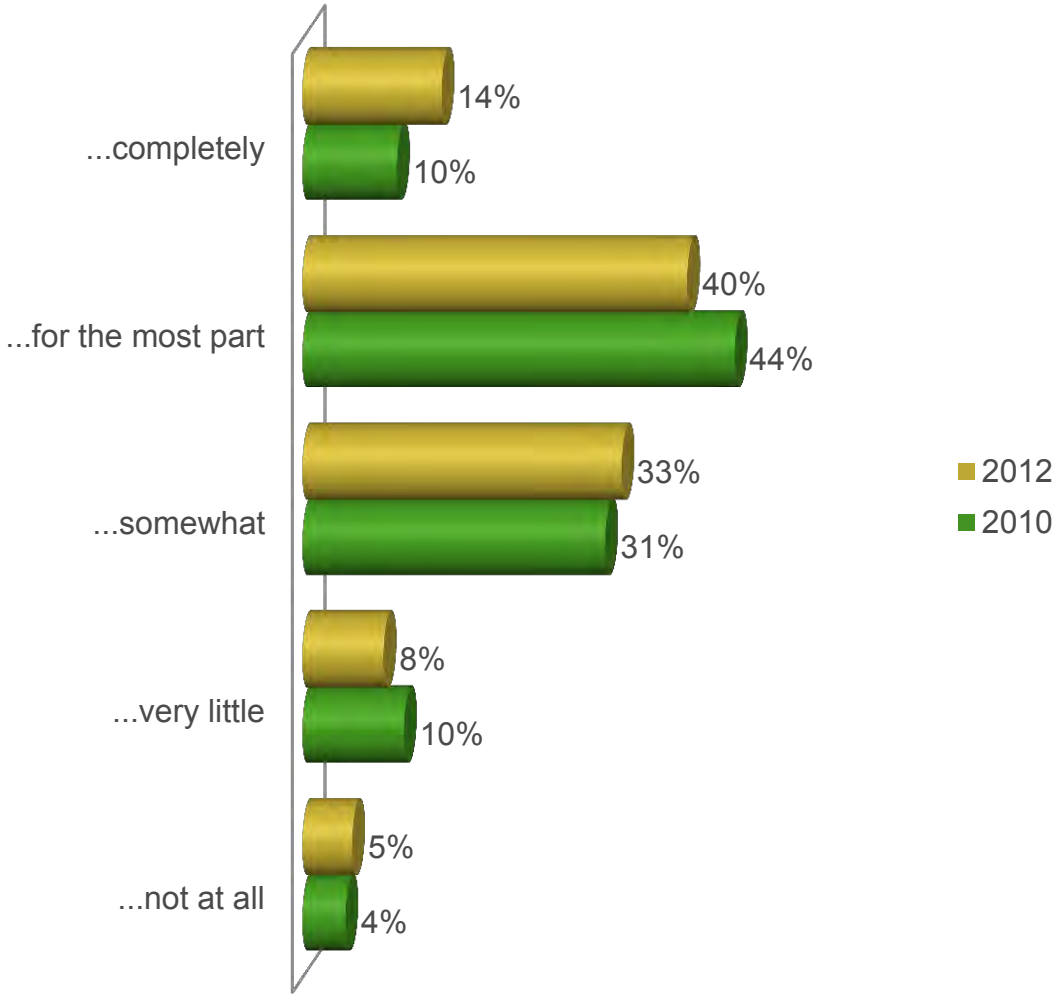
Consumers worry organic food is processed on the **same equipment** as conventional items.

They question whether big companies are influencing USDA Organic labeling criteria, making it **less stringent**.

> *I worry about the machinery – I doubt they have organic and non-organic machines. I think that's a problem and it gives me less faith in companies that make both" " –* Carita, (Inner Mid-Level)

As a result, most consumers only moderately trust the USDA Organic certification

Only 40% trust the USDA organic label "for the most part"...



> *People get paid a lot of money to come up with certifications"* – Ayanda, (Inner Mid-Level)

> *You have to take these labels for what they're worth and hope for the best."* – Jeff, (Outer Mid-Level)

And, the rise of processed organic "junk" foods is weakening the link between healthy and Organic

Consumers today are **less likely to assume** a product is healthy simply because it carries the Organic label.

The Organic certification **loses appeal** on products full of sugar, corn syrup and unidentified ingredients.

Organic **does not have the power** to transform processed treats into health foods.

Consumers don't want to pay the **organic premium** for foods that are not healthy anyway.

> *Junk is junk, why pay extra for organic junk – it's not good for you whether it's organic or not.*" – Josephine, Seattle (Outer Mid-Level)

While the meaning of Organic has weakened, Natural is experiencing renewed significance

The term "natural" is more meaningful to consumers today than it was in 2010:

Consumers increasingly desire foods that are **less processed** with **clean ingredient** lists (56% say natural foods contain nothing artificial)

They want **fresh**, **real** foods (46% say natural foods are real and 47 % say they are pure)

However, natural as a **marketing term** remains vague and **unappealing** to consumers.

"*Natural is the fewest ingredients with the least processing. It's the simplest form – untainted."* – Kass (Outer Mid-Level)

"*Natural means that I could make the same product in my kitchen or grow."* – Rebecca (Inner Mid-Level)

PRODUCT CATEGORY ANALYSIS

Gateway categories into Organics are the same for all Organic users

Consumers across all segments – core, mid-level and periphery – try

fresh vegetables, **fresh fruits** and **milk**

first in organics.

24%

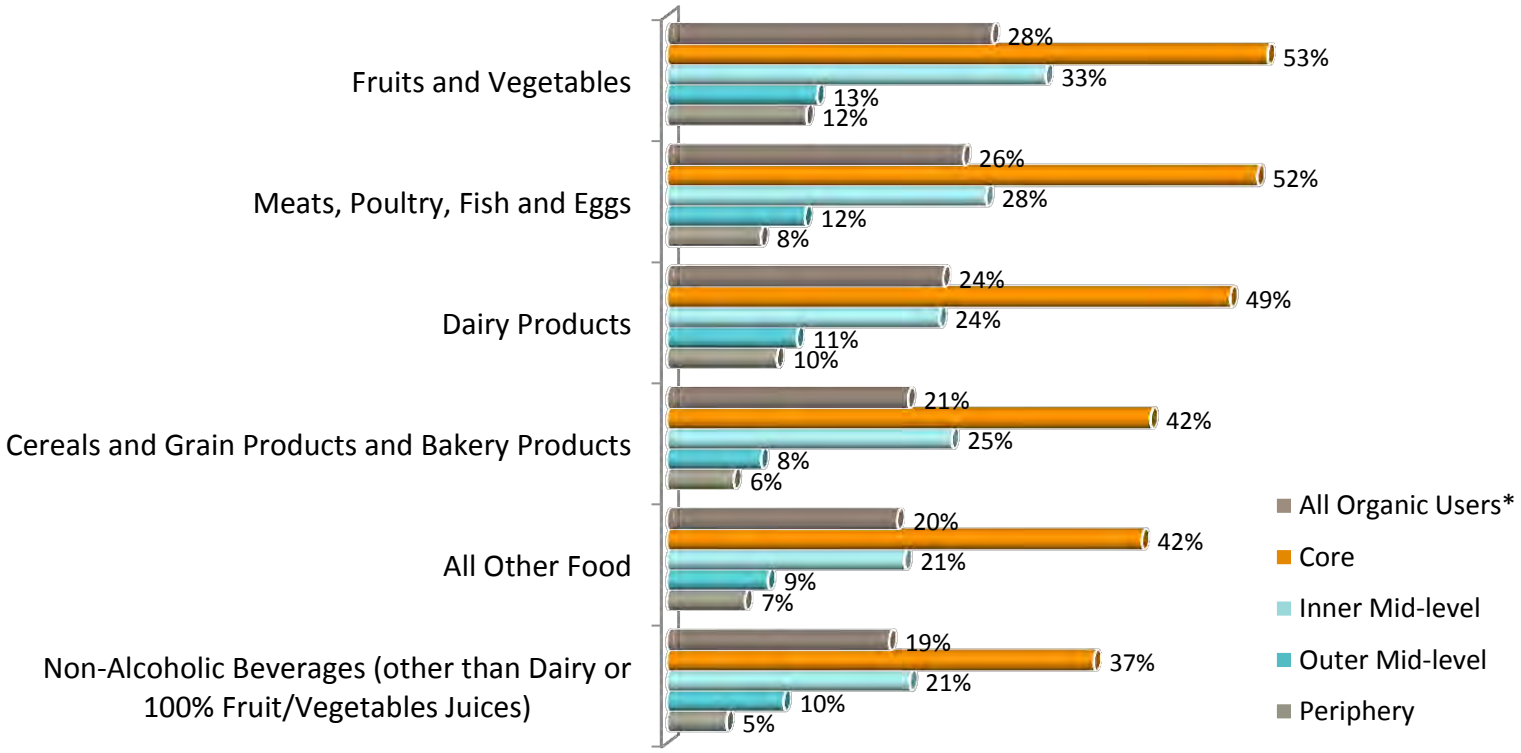
17%

12%

Percentage of all organic users that tried the above categories first

As consumers become more involved in Organic and Natural foods, they prioritize spending on produce, meat and dairy

Inner Mid-level consumers spend far more on Organic and Natural products than do the Outer Mid-level or Periphery segments



Portion spent in the last 30-days on organic and natural foods and beverages.

Even as consumers buy more processed Organics, whole foods are consumers' top priority, but price is a barrier

Consumers consider whole foods the most nutritious organics.

WHOLE

Consumers want to eat more organic meat but price is a major obstacle.

Increasing numbers of consumers are experimenting with reasonably priced organic cereals and breads.

PACKAGED

Consumers use boxed, canned and frozen organics in variable quantities and with sporadic frequency. These items are most susceptible to price comparisons and sale shopping.

ESTABLISHING TRUST

Establishing a trusting relationship with consumers is challenging in the current economic climate

Our research reveals a population of consumers who are:

bewildered and **mistrustful** of big business and government

worried that **profits** are valued above health

blaming the food industry for **obesity** and **diabetes**

Consumers believe large companies can help alleviate their concerns by providing organic and natural foods at prices they can afford.

> *I'm wary of big companies. They're out for profit. They don't care about me."* – Carl, (Inner Mid-Level)

> *Big Companies have to really work a lot harder to gain my trust."* – Carrie, (Inner/Outer Mid-Level)

GMOs are an issue that is testing the limits of consumer trust

The popular media is shaping the discussion around GMOs, leaving consumers to ask **why large companies are so silent?**

Consumers across segments are **confused** and want to know:

- What's a GMO?
- What foods are they in?
- Are they safe?

Over 50% of all consumers look for organic foods because they don't contain GMOs.

The longer companies avoid discussion of GMOs the more wary consumers will become. Food companies need to be an active participant in the conversation

Non GMO product display at Natural Foods Expo 2012 (from Wholesoy, Flickr.com)

In this climate, retailers take on key roles as docents for consumers

59% of consumers buy their organic foods at **regular grocery stores**

Consumers look to these larger retailers for help understanding **confusing** information around organics

They expect reputable retailers to **vet products** for them

Consumers are more likely to **believe** the claims of products sold at stores they trust, they **assume** these stores don't sell brands that mislabel and mislead

If consumers truly trust a retailer, they are more likely to assume the store does not sell products that mislabel and mislead.

Establish trust by aligning yourself with the broader set of values that Organic now signifies

Organic no longer simply means a "better for me" product. It now signifies a **"better we"** – as people and a planet.

- Food makes you healthy not sick
- Children are not obese
- Workers are paid a living wage
- Employees are empowered
- Science benefits more than the bottom line
- Animals are raised humanely

Developing organic products is **necessary, but not sufficient** to convince consumers you share their wider values.

To be truly relevant in Organics, you must enter the "better for we" space

Parting Thoughts: Key Takeaways

- The meaning of "Organic" is becoming more **diluted**.
- While over half of consumers are aware of government regulations controlling organic labeling, the USDA Organic label only generates moderate levels of **trust**.
- Compared with "Organic," "Natural" is about **simple**, **real foods**.
- Natural and Organic **meats** are an area of growing consumer interest.
- The path to adoption of organic products is usually through produce and other **nutrient dense foods**.
- Mainstream **retail channels** continue to rise in importance as sources for organic foods and beverage.
- Demonstrate your commitment to a **"better we"** to increase your relevance to consumers and gain their trust.

Hungry For More?

Contact us to learn more about the full report or just reach out with any questions you may have about this report or Hartman's other syndicated offerings.

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